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THE INQUIRING MIND

No pleasure is comparable to the standing upon the vantage ground of truth.

—Francis Bacon (1561–1626).

Now, who shall arbitrate ?
Ten men love what I hate,
Shun what I follow, slight what I receive.
Ten, who in ears and eyes
Match me : we all surmise
They, this thing, and I, that : whom shall my soul believe ?

—Robert Browning (1812–1889).

An apparent paradox is found throughout medical life : from student to venerable practitioner an inquiring mind is postulated or professed, and yet all medical ages have a most persistent desire to cling to authority. The dogmatism of Galen dominated medical thought for a thousand years, until the inquiries of minds such as those of Vesalius and William Harvey opened the way to the extension of knowledge. The wish for authority did not die, however, and there were bitter struggles between rival schools contending for dominance—the humoralists versus the solidists, the Brunonians versus the Broussaisians, the homœopaths versus the regulars. Today we read of these dissensions with complacent tolerance, secure in our thoughts that medicine is now firmly based on scientific truth and that the enormous extensions of knowledge provided by the biological sciences within the last century have revolutionized medical philosophy. It could be argued, however, that the desire for authority is still with us, and that one of the fundamental problems of medicine today is the multiplicity of authorities ; there is a babel of voices claiming our attention.

Medical students of today approach their clinical training after long exposure to the basic and biological sciences at both school and university. Their minds should be trained in the methods of science, they should be capable of critical appraisal, of logical deduction ; they should be searchers after truth. With depressing regularity, however, dogmatism is preferred. Popular is the professor who writes and prescribes his own textbook, more loved is the lecturer who distributes typed and tabulated summaries.

Oh ! rather give commentators plain
Who with no deep researches vex the brain :
Who from the dark and doubtful love to run,
And hold their glimmering tapers to the sun.

—George Crabbe (1754–1832).

It is possible (and this experiment is recommended to all clinical teachers) to make statements which are physiologically or biochemically or even anatomically absurd, which are not challenged. It is interesting further to observe that, when the deception is confessed, the students' reaction is more one of annoyance with the teacher than with themselves.

Lies the fault in the students or in their teachers, or in both? There is no lack of authoritative pronouncement on this problem. The humanists decry the concentration on science and prescribe large doses of "cultural" subjects. The curriculum manipulators advocate "integration" and damn "departmentalism". The clinicians blame the academics and hint that all would be well if the sciences were taught by themselves, while, oddly enough, the pre-clinicians suggest that their efforts are lost because few clinical teachers apply the knowledge and precepts of the basic sciences. It is notable that, in the voluminous writing and discussion on the problems of medical education, there is a welter of opinion and impression, and a dearth of studied information on methods of learning and of teaching.

The practitioners of medicine of today face the appalling problem of attempting to apply their knowledge, whilst simultaneously revising and extending it, in the light of a torrential flood of medical information, some true, much false. The natural desire for authoritative direction in this dilemma has had various results. The multifarious attempts at post-graduate instruction at every level are one consequence. Perhaps it is not surprising that the post-graduate student is found to resemble his undergraduate junior in tending to value most highly those instructors whose message is comfortably dogmatic. It is easier for the heavily-burdened mind to accept than to question. Another consequence of our situation is the rash of gaily-coloured publications providing potted information. Such distillation would be admirable if only the critical point at which truth emerges was known; but, alas, it is not. Many, realizing that no man can read, even less digest, all that is printed, limit their attention to one field. This has produced, for their convenience, journals of increasingly narrow scope, and the total output of literature is thus steadily increased. Another consequence is the rapid spread of the condition aptly termed *Monsterkongresskrankheit*, in which it is believed that truth can be characterized by collective action. Others of us, in our predicament, fall victim to authoritarianism in a new guise, the cult of the novel. Energetically encouraged by pharmaceutical interests, we come to accept that which is new as better or more true. The rapid emergence of this philosophy in a profession otherwise innately and rightly conservative can be explained only as an inverted form of the desire for authority—a recognition that we should always be questioning, but an acceptance of an easy but false standard of judgement. Once again there is no lack of voice from those who would solve our difficulties. Pronouncements emanate from all quarters within the profession and also, most ominously, from without, ranging from the paramedical scientists, who see little difference between the practice of medicine and the activities of their laboratories, to governments which have the delusion that regimentation removes problems.

Surely it is certain that in this complexity there is no simple solution and no single panacea. One certainty is that scientific knowledge, despite phenomenal advance, does not yet more than touch the fringes of the almost inconceivable complexity of the human being in health or disease. While this remains so, and it will for very long, the actual practice of medicine must remain a skilled art, the application of experienced judgement, with only a slender raft of facts to support us in a vast sea of ignorance. Osler's comment is no less true today:

> Variability is the law of life. As no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions we know as disease. This is the fundamental difficulty in the education of the physician and one which he may never grasp or he takes it so tenderly that it hurts, instead of boldly accepting the maxim of Bishop Butler, more true of medicine than of any other profession, "Probability is the guide of life". Surrounded by people who demand certainty, and not philosopher enough to agree with Locke that "probability supplies the defect of our knowledge and guides us when that fails and is always conversant about things of which

we have no knowledge ", the practitioner too often gets into a habit of mind which resents the thought that opinion, not full knowledge, must be his stay and prop. There is no discredit, though there is at times much discomfort in this everlasting *perhaps* with which we have to preface so much connected with the practice of our art.

It is incumbent on all of us, particularly perhaps our colleges, whether of general practice or of specialism, and our universities with their large number of recently created clinical departments, to hold fast to that which has been truly shown to be true, to resist the temptation to take refuge in apparent authority, and to question always, with a truly open and inquiring mind.

H. N. ROBSON.

AUTOIMMUNITY IN HUMAN THYROID DISEASE[1]

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SUMMARY

The occurrence of circulating antibodies to thyroid antigens was investigated by the tanned-red-cell hæmagglutination (T.R.C.H.) and complement fixation (C.F.) tests, whole thyroid extract being used as antigen. Whole thyroid was found to be equivalent to thyroglobulin as an antigen in the T.R.C.H. reaction.

In Hashimoto's thyroiditis and myxœdema, the incidence of positive T.R.C.H. reactors (titres greater than 100) was over 70% and in thyrotoxicosis 47%.

In thyrotoxicosis, the incidence of positive reactors was influenced by therapy (radio-iodine or surgery), but was independent of age, sex, toxicity and the type of goitre.

The present study indicates that "leakage" of thyroid components with immunological response occurs frequently in thyroid disorder and occasionally in health, but *per se* has no pathogenic significance.

It is postulated that, under certain circumstances, perhaps associated with weakness of immunological homœostasis, immunologically competent cells reaching and reacting with thyroid tissue give rise to damage, thus rendering more antigen accessible and so leading to "colonization" of the tissue with lymphoid cells and self-perpetuating disease

THERE is impressive clinical and experimental evidence which suggests that immunological mechanisms are implicated in the pathogenesis of chronic lymphadenoid (Hashimoto's) thyroiditis. Thus, the gamma globulin content of the serum is raised and circulating antibodies are present which react specifically with extracts of human thyroid (Roitt and Doniach, 1958). Experimentally, Witebsky *et alii* (1957) found that thyroiditis resembling Hashimoto's disease could be induced by inoculation of an animal with homologous but not heterologous thyroid tissue, and even with the animal's own surgically removed hemithyroid. This experimental auto-immune thyroiditis resembled the human counterpart, in that the gland showed severe damage and was densely infiltrated by lymphocytes and plasma cells, and antithyroid antibody was present in the blood in high titre.

The present study was designed to investigate the nature and significance of auto-immunity in human thyroid disease.

MATERIALS AND METHODS

Serum from subjects without clinical evidence of thyroid disease was obtained from normal blood donors attending the Red Cross Blood Transfusion Service and the biochemistry department of the Royal Melbourne Hospital, and from patients attending the Clinical Research Unit. Serum from patients with thyroid disease was obtained from the thyroid clinic of the Royal Melbourne Hospital, and through the courtesy of Dr. F. F. Rundle from The Royal North Shore Hospital of Sydney. Diagnosis was established on clinical assessment and radio-iodine uptake studies. A few sera were also sent by physicians and surgeons in other centres. The immunological techniques employed to detect antibody to human thyroid and other tissue antigens included a modification of the tanned-red-cell hæmagglutination reaction of Boyden (1951) and the complement fixation reaction (Gajdusek, 1958).

Tanned-Red-Cell Hæmagglutination Reaction (T.R.C.H.)

Preparation of Sensitized Tanned Cells.—Thrice-washed normal sheep cells (collected in Alsever's solution) were made up to a 5% suspension and mixed with an equal volume of a 1/25,000 dilution of freshly prepared tannic acid solution. Both the cell suspension and the tannic acid solution were made up in phosphate-buffered saline (pH 7·2). The mixture was incubated for 25 minutes at 37° C., and the cells were washed once in the same buffer. One millilitre of packed tanned cells was mixed with 20 ml. of a 1 : 10 dilution of thyroid extract (*vide infra*) prepared in phosphate-buffered saline at pH 6·5, and incubated for 45 minutes at 37° C. The cells were then washed twice in a barbital acetate buffer at pH 5·7, prepared according to Michaelis (1931). The cells were finally made up to a 1% suspension in diluent.

[1] Received on January 22, 1960.

[2] Assistant Physician to the Clinical Research Unit.

[3] Research Officer.

[4] Working with the aid of a grant from the National Health and Medical Research Council of Australia.

Diluent.—This was 1% normal rabbit serum in phosphate-buffered saline at pH 7·0; the serum had been previously inactivated for 30 minutes at 56° C., and then adsorbed for 20 minutes at room temperature with an equal volume of packed normal sheep cells.

Preparation of Antigen.—Thyroid tissue was obtained from cadavers not more than 16 hours after death. The tissue was washed in physiological saline, minced with scissors and ground in a mortar with sterile sand. The tissue was then suspended in phosphate-buffered saline (pH 7·0) to make a 10% w/v suspension. This was lightly centrifuged to remove the sand and larger tissue particles, and the supernatant was spun at 5000 r.p.m. in a "Spinco" ultracentrifuge (rotor 30) for 15 minutes. The resulting supernatant, referred to as the "thyroid extract", was stored in the frozen state and thawed as required.

Tanned sheep cells were also coated with thyroglobulin, prepared by the method of Derrien *et alii* (1948), instead of thyroid extract. The thyroglobulin was used at a concentration of 0·25 mg. per millilitre in phosphate-buffered saline at pH 6·5. One millilitre of packed tanned sheep cells was sensitized with 20 ml. of this concentration of thyroglobulin.

A comparison of titres, with the use of whole thyroid extract and thyroglobulin as antigen, suggested that the effective antigen in the whole thyroid extract was in fact thyroglobulin (see Table III).

Sera.—All sera were diluted 1 : 10 in physiological saline, inactivated for 30 minutes at 56° C., and adsorbed for 20 minutes at room temperature with a half volume of packed normal sheep cells.

Titrations.—Doubling dilutions of sera were prepared in diluent in 0·25 ml. amounts, and 0·25 ml. of 1% sensitized tanned sheep cells was added. The titrations were left overnight at room temperature before being read. The titre was expressed as the reciprocal of the serum dilution giving the usual "+" degree of partial agglutination.

Controls.—A number of control tests were included with each fresh batch of sera and sensitized cells. The cells were tested for stability by the addition of one volume of 1% cells to one volume of diluent. One volume of 1 : 10 diluent of each serum was mixed with one volume of a 1% normal sheep cell suspension and with one volume of a 1% tanned sheep cell suspension; this was done to ensure that adequate adsorption had been carried out. Tests for the specificity of the hæmagglutination reaction were carried out as follows: 0·25 ml. of a 1 : 10 serum dilution was mixed with 0·25 ml. of a 1 : 5 or 1 : 10 dilution of thyroid extract (prepared in diluent), and was allowed to stand for one hour at room temperature before the addition of 0·25 ml. of 1% sensitized tanned sheep cell suspension. The results were read after the tests had remained overnight at room temperature.

Complement Fixation Reaction

The C.F. technique and preparation of human tissue antigens were as described by Gajdusek (1958). Antigens were prepared from toxic thyroid gland obtained surgically, and from non-toxic thyroid, liver and kidney obtained from cadavers. Prior extraction of thyroglobulin was found not to influence the antigenicity of toxic thyroid glands in the C.F. reaction.

RESULTS

The results of T.R.C.H. and C.F. tests in thyroid and non-thyroid disease are presented as case distribution tables for successive increments of serum titre from negative to greater than 20,000 (Tables IA and IB), and graphically for the T.R.C.H. tests (Figure I). Sera reacting to a titre of less than 20 in the T.R.C.H. test and less than 4 in the C.F. test were regarded as giving negative results. T.R.C.H. titres of 20 to 100 and C.F. titres of 4 to 8 were regarded as weakly positive and of uncertain significance.

Non-Thyroid Disease—Blood Donors and Hospital Subjects

Of 168 blood donors tested, 9% had serum T.R.C.H. titres of more than 20, and 4% had titres of more than 100; 52 were tested by C.F., toxic thyroid being used as antigen, and 8% reacted to titres of 4 to 8 and 2% to titres of more than 8. Positive reactions could not be correlated with the age and sex of donors, or with the numbers of blood donations given. There was no correspondence between positive reactors in the T.R.C.H. and C.F. tests.

A number of sera from patients without overt thyroid disease were analysed. One group comprised 200 sera drawn from patients attending this Unit and may have been biased towards immunological anomalies, although sera from patients with systemic lupus erythematosus and liver disease were considered separately. In 14 of the 200 subjects (7%), 12 of whom were females, the T.R.C.H. titre was more than 100. The diagnoses in the "positive" cases were as follows: cholelithiasis, sprue and osteomalacia, nephrotic syndrome (two cases), primary biliary cirrhosis, idiopathic thrombocytopenic purpura, suspected systemic lupus erythematosus, macroglobulinæmia, atrophic gastritis (two cases), recurrent boils, myocardial infarction (two cases) and myelitis. A second group of 94 sera was collected at random from the biochemistry department of the hospital; of these, five reacted to titres of more than 100, the diagnoses being congestive cardiac failure (three cases), gout and infectious hepatitis. There was an incidence of eight weakly positive C.F. reactions (titre 4 to 8) with toxic thyroid antigen in 52 hospital subjects without overt thyroid disease, and in one of the 52 cases the C.F. titre was more than 8.

Positive results to the T.R.C.H. test (titre more than 100) were obtained from 15% of 33 patients with systemic lupus erythematosus and in 16% of 31 patients with active chronic and lupoid hepatitis. This may suggest a

surprisingly high incidence of inapparent thyroid disease in systemic lupus erythematosus and hepatitis; but this reactivity was possibly adventitious and due to the formation of gamma globulins with a non-specific affinity for host constituents, whether thyroglobulin or the mixed tissue antigens of the auto-immune complement fixation reaction (Gajdusek, 1958; Mackay and Larkin, 1958).

The possibility was considered that high-titre T.R.C.H. reactions, particularly in non-thyroid disease, might be due to antibodies reacting with antigens not specific to the thyroid gland. However, when T.R.C.H. tests were performed with 28 high-titre sera using cells coated with whole human liver extract as antigen, negative results were obtained.

Thyroid Disease

The high incidence of T.R.C.H. and C.F. reactions in Hashimoto's disease and myxœdema (Tables IA and IB) agrees with other published observations. Two patients who were treated surgically had lower serum titres. Of 104 thyrotoxic patients, the T.R.C.H. titre was more than 20 in 72%, more than 100 in 47%, and more than 10,000 in 12%. Only 14% of 42 patients with non-toxic goitre showed T.R.C.H. titres of more than 100. Positive results were also obtained in acute thyroiditis and thyroid carcinoma.

Reactivity in Thyrotoxicosis

In thyrotoxicosis, the overall incidence of positive T.R.C.H. titres (more than 100) was 47%. Sex, age, nodular goitre and toxicity could not be correlated with positive results, since titres of more than 100 were obtained in 52% of the males, in 41% of subjects aged under 40 years, and in 50% of those aged over 40 years, in 48% of patients with diffuse goitre, in 45% of patients with nodular goitre, in 44% of currently toxic patients, and in 45% of currently non-toxic patients. However, therapy did influence antibody titres: patients treated with radio-iodine showed a lower incidence of positive results in T.R.C.H. tests, whereas surgically treated patients showed a lower incidence of

TABLE IA

Tanned-Red-Cell Hæmagglutination Titres of Serum in Normal Subjects and in Thyroid and Non-Thyroid Disease: Distribution

Subjects and Diagnosis	Number	T.R.C.H. Titre					Titre Greater than 100 (Percentage)
		0 to 20	20 to 100	100 to 1000	1000 to 10,000	Over 10,000	
Normal blood donors	168	153	8	4	3	0	4
Thyroid disease:							
Hashimoto's disease	11	1	2	2	1	5	72
Myxœdema	5	0	1	1	0	3	—
Thyrotoxicosis	104	32	25	23	11	13	47
Non-toxic goitre	42	28	8	1	4	1	14
Acute and subacute thyroiditis	9	4	2	2	0	1	—
Carcinoma	3	1	1	0	0	1	—
Non-thyroid disease:							
Unit cases	200	171	15	10	2	2	7
Hospital cases	94	81	8	5	0	0	5
Systemic lupus erythematosus	33	25	3	5	0	0	15
Hepatitis:							
Active chronic	31	22	4	4	1	0	16
Nutritional	12	9	3	0	0	0	—
Hæmochromatosis	7	5	0	2	0	0	—
Rheumatoid arthritis	27	22	3	1	1	0	7

TABLE IB

Complement-Fixation Titres of Serum with Liver and Kidney and with Toxic Thyroid Antigen: Case Distribution

Subjects and Diagnosis	Number	C.F. Titres						Greater than 8 (Percentage)
		Liver and Kidney Antigen			Toxic Thyroid Antigen			
		Less than 4	4 to 8	More than 8	Less than 4	4 to 8	More than 8	
Normal blood donors ..	52	51	1	0	47	4	1	2
Hospital patients	61	61	0	0	52	8	1	2
Hashimoto's thyroiditis ..	6	6	0	0	2	1	3	—
Thyrotoxicosis	79	74	5	0	36	20	23	30
Non-toxic goitre	14	13	1	0	11	1	2	14

positive results in C.F. tests compared with the entire group (Table II).

There was only a limited correlation between T.R.C.H. and C.F. titres in thyrotoxicosis (Figure II), sera reacting strongly in one test often failing to react in the other. The T.R.C.H. and C.F. reactions do in fact depend on separate antigens (*vide infra*). Only 13 of the 104 sera gave negative responses to both tests.

Thyroglobulin versus Whole Thyroid Extract as T.R.C.H. Antigen

T.R.C.H. tests were performed on 173 sera to compare whole thyroid extract and isolated thyroglobulin as antigen (Table III). The titre of 51 of these sera was less than 20; in these, the titres with the two antigens either were identical or differed by one dilution only. The remaining 122 sera, which were from

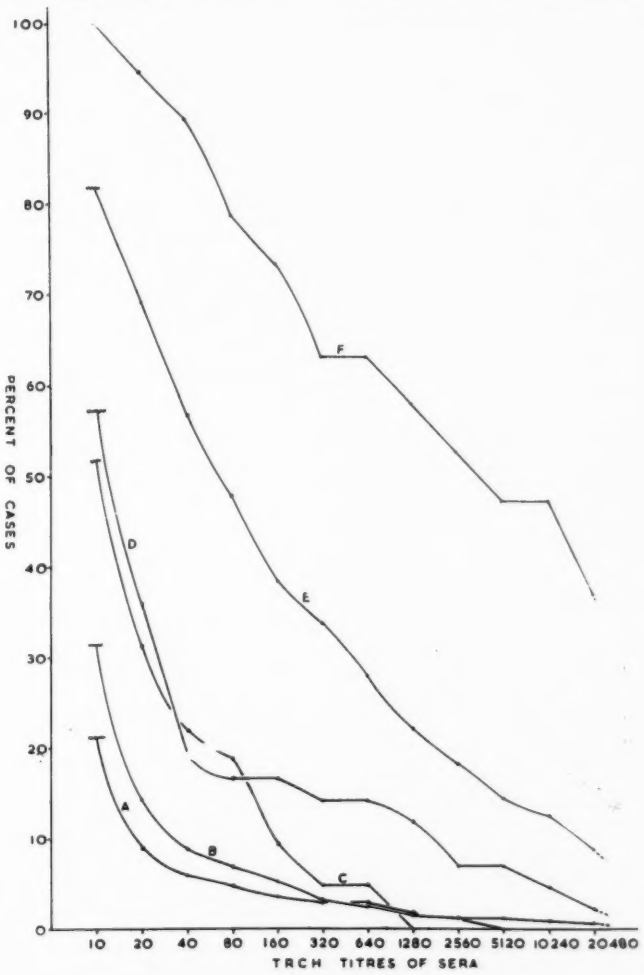

FIGURE I

Incidence of thyroglobulin antibody (T.R.C.H. reaction) in normal subjects and in disease. "Percentage of cases" (ordinate) is that percentage in which titres of, and greater than, the corresponding point on the abscissa were obtained. A, normal blood donors; B, hospital and Unit cases; C, systemic lupus erythematosus, active chronic and lupoid hepatitis; D, non-toxic goitre; E, thyrotoxicosis; F, Hashimoto's thyroiditis and myxœdema. The curves for systemic lupus erythematosus and hepatitis were similar and hence were combined

patients with and without thyroid disease, reacted to titres of more than 20; of these, titres were identical in 22 cases, and differed by one dilution in 62 cases, by two dilutions in 21 cases, and by three dilutions in nine cases. These differences were considered to be within experimental variation. However, T.R.C.H. titres differed by more than three dilutions in eight sera; five sera were from patients with thyrotoxicosis in which the titre with whole thyroid extract was greater than the titre with thyroglobulin, whereas in sera from three patients with Hashimoto's thyroiditis the reverse was true.

TABLE II

Incidence of Positive Results to T.R.C.H. and C.F. Tests in Relation to Treatment in Thyrotoxicosis

Treatment Category	T.R.C.H. Test		C.F. Test	
	Number of Cases	Titre Greater than 100 (Percentage)	Number of Cases	Titre Greater than 8 (Percentage)
All cases of thyrotoxicosis ..	104	47	79	30
Untreated by radioiodine or surgery	34[1]	60	24	40
Radioiodine ..	44	35	33	36
Surgery	26	50	22	9

[1] Certain of these subjects received carbimazole.

Thus satisfactory T.R.C.H. results were obtained by the use of whole thyroid extract as antigen, rather than thyroglobulin, which is more difficult to prepare. However, in some cases of thyrotoxicosis there may have been an effective antigen other than thyroglobulin attached to the tanned cells.

Reactivity of High-Titre Sera with Autologous Thyroid Antigen

The phenomenon of non-reactivity of autoimmune antibody, previously shown with autologous antigens such as liver and kidney by Mackay and Larkin (1959), was not observed with thyroid antibodies. Four sera which gave high T.R.C.H. titres with homologous antigens were tested with the patients' own (autologous) thyroid as the source of antigen. In two of these cases the thyroid was totally non-antigenic, and in two the sera reacted equally with autologous and homologous antigen. Witebsky *et alii* (1958) have made a similar observation. Seven high-titre C.F. sera were similarly tested; in six cases the thyroid was non-antigenic, and in one case the serum reacted equally with autologous and homologous antigens.

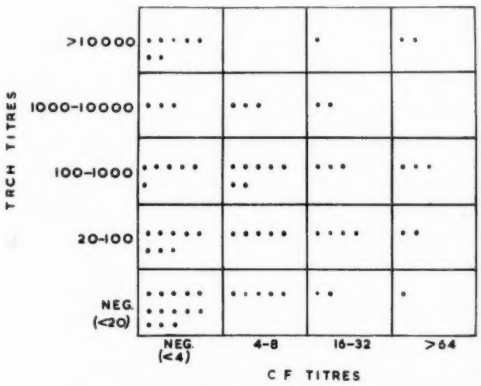

FIGURE II

Comparison of T.R.C.H. and C.F. titres in thyrotoxicosis

Coexistence of Thyroid Disorder with Systemic Disease

In 11 cases observed at this hospital (Table IV), systemic disease, particularly systemic lupus erythematosus, rheumatoid arthritis and chronic hepatitis, coexisted with thyroid disease (thyroiditis, hypothyroidism and thyrotoxicosis). In particular, two cases of hypothyroidism and three cases of thyrotoxicosis occurred in association with systemic lupus erythematosus. The

TABLE III

Comparison of Whole Thyroid Extract with Thyroglobulin as an Antigen in the T.R.C.H. Test

Result	Number of Sera	Difference in T.R.C.H. Titre with Thyroid Extract and Thyroglobulin as Antigen—Number of Sera				
		Titre Identical	Titre Differing by			
			One Dilution	Two Dilutions	Three Dilutions	More than Three Dilutions
Negative response (titre less than 20) ..	51	38	13	0	0	0
Positive response reactions (titre more than 20)	122	22	62	21	9	8[1]

[1] See text.

TABLE IV

Systemic Manifestations in Patients with Thyroid Disease

Subjects Studied[1]	Thyroid Disease	Systemic Disease	C.F. Titre[2]		T.R.C.H. Titre
			L	T.T.	
L.C.	Hashimoto's thyroiditis	Rheumatoid arthritis, angioneurotic œdema	<4	32	20,000
E.A.	Hashimoto's thyroiditis	Active chronic hepatitis, gastric ulcer	<4	512	960
P.B.	Hashimoto's thyroiditis	Scleroderma with renal disease	—	—	—
N.C.	Subacute thyroiditis	Rheumatoid arthritis	<4	—	960
R.S.	Myxœdema	Systemic lupus erythematosus	—	—	—
D.M.	Myxœdema	Systemic lupus erythematosus	<4	<4	—
R.D.	Myxœdema	Rheumatoid arthritis, result of L.E. test positive	8	—	—
J.T.	Thyrotoxicosis	Systemic lupus erythematosus	—	—	—
H.T.	Thyrotoxicosis	Systemic lupus erythematosus, drug eruptions	<4	<4	10
J.K.	Thyrotoxicosis	Systemic lupus erythematosus, chronic hepatitis, chronic ulcerative colitis	<4	<4	40
L.W.	Thyrotoxicosis	Thrombocytopenic purpura	32	16	15

[1] All subjects were females. Certain of them were observed prior to availability of serological tests.
[2] L., liver antigen; T.T., toxic thyroid antigen.

coexistence of Paget's disease, nephrosis and adrenal atrophy with thyroiditis is well documented (see Roitt and Doniach, 1958). The implication is that a more general failure of immunological homœostasis may occasionally supervene in thyroid disorders.

DISCUSSION

Our investigations have shown that antibodies to thyroid components are characteristic of chronic thyroiditis and are detectable also in other types of thyroid disease; these findings are in general agreement with other published observations on auto-immunity in thyroid disease. Present knowledge (see Roitt and Doniach, 1958, 1959; *The Lancet*, 1958; Ovary *et alii*, 1958; Owen, 1958; Anderson *et alii*, 1959*a*, 1959*b*; Belyavin and Trotter, 1959; Blizzard *et alii*, 1959; Cline *et alii*, 1959) may be summarized as follows:

1. Circulating antibodies to at least three thyroid components may be present independently and in differing titre: (*a*) an antithyroglobulin antibody, detectable as a precipitin by complement fixation, by the highly sensitive tanned-red-cell hæmagglutination reaction and by passive cutaneous anaphylaxis of the guinea-pig; (*b*) a C.F. antibody reactive with a cellular antigen, possibly microsomal, which is present particularly in toxic glands; (*c*) antibodies to the mixed cellular antigens of the A.I.C.F. reaction.

2. Circulating antibody, extracted from the serum of patients with Hashimoto's disease and labelled with fluorescein, will combine *in vitro* with antigen in slices of thyroid gland and regional lymph nodes (White, 1957; Hiramoto *et alii*, 1958); the independence of the thyroglobulin and microsomal antibodies is also demonstrable by this technique.

3. Circulating antibodies to thyroid gland components are demonstrable to a varying degree in most types of thyroid disease. Their incidence is greatest, and the titres are highest, in Hashimoto's disease, with next in frequency adult myxœdema (Owen and Smart, 1958); lower titres may be encountered after thyroidectomy or when the gland is replaced by fibrous tissue. The incidence of thyroid antibody is surprisingly high in thyrotoxicosis; we found a lower incidence of positive T.R.C.H. titres after radio-iodine therapy, and a lower incidence of positive C.F. titres after surgery. Antibodies are also detectable in other thyroid diseases including carcinoma (Stuart and Allan, 1958; Doniach *et alii*, 1958).

4. Positive skin reactions to thyroid extract have been observed in patients with Hashimoto's disease and myxœdema (Buchanan *et alii*, 1958).

The presence of circulating antithyroid antibody led to the supposition that chronic thyroiditis might depend upon auto-immune mechanisms for its perpetuation (*Brit. med. J.*, 1958). It was assumed that initial and usually subclinical damage to the thyroid resulted in escape of thyroid constituents. This in turn caused stimulation of antithyroid antibody formation and a further damaging immune reaction with thyroid antigen. Fresh antigen would thus be liberated, with the initiation of a "vicious circle" or "chain reaction"; this would culminate in destruction of the gland, a process which we have termed "autoclasia" (Mackay *et alii*, 1959). Although this general concept of thyroiditis is accepted, certain relevant problems invite further discussion, as follows:

(1) The factors responsible for initiating lymphadenoid thyroiditis are unknown. Occasional patients with chronic thyroiditis have had antecedent thyrotoxicosis; but a past

history of thyroid-damaging disease is usually indefinite or lacking (Hazard, 1955; Wooiner *et alii*, 1959). Certainly there is seldom a clinical episode comparable with the intense immunizing procedure used to produce experimental thyroiditis (Witebsky *et alii*, 1957). Moreover, gross thyroid damage may occur, as during radio-iodine therapy, with no antibody response. It is of interest that Beare (1958) found that patients with Hashimoto's disease had increased vulnerability to various diseases, suggesting " pathological sensitivity ".

(2) There exists the problem of differentiating " immunization " associated with non-pathogenic circulating antibody, with or without mild lymphoid change in the thyroid gland, from " immunization " associated with autoclastic thyroiditis of the Hashimoto type. In other words, the occurrence of antithyroid antibody in thyrotoxicosis and thyroid cancer strongly suggests that auto-immunization is a result rather than the cause of thyroid dysfunction or damage, since only quantitative differences exist between the serological reactivity in thyroiditis and thyrotoxicosis and even in subjects without overt thyroid disease. The reactivity which we detected in normal blood donors and in patients with systemic lupus erythematosus and chronic non-alcoholic liver disease (see also Goudie *et alii*, 1959) could reflect lymphoid invasion of the thyroid gland which occurs particularly in elderly women (see Doniach and Roitt, 1957), although histological material from our own cases did not entirely support this concept. Alternatively, the antithyroid reactivity of the serum in systemic lupus erythematosus and chronic hepatitis may be entirely adventitious, since these conditions are characterized by an excessive production of globulins with a broad immunological affinity for human tissue proteins.

(3) Cytopathogenic effects have been accepted only for auto-antibodies which react with blood cells. Although sera from patients with thyroiditis have shown some cytotoxic effects (Pulvertaft *et alii*, 1959), high titres of thyroid antibody may be present with only minimal evidence of thyroid damage. This uncertainty regarding the cytopathogenicity of circulating antibody has resulted in attention being shifted to a different type of immune reactivity. This type of activity, which is responsible for tuberculin hypersensitivity and other types of delayed hypersensitivity including the homograft rejection phenomena, is not associated with circulating antibody, and, being intimately associated with lymphoid cells, is sometimes referred to as " cell-borne antibody "; however, we prefer the non-committal term " immunologically competent cells " (I.C.C.) in reference to this type of immune reactivity. Although the present trend of opinion is to implicate such immunological activity in the pathogenesis of thyroiditis and other visceral immunopathies (Mackay *et alii*, 1959; Brent and Medawar, 1959), the hypothesis suffers from its convenience, since techniques are lacking to establish experimentally the immunological competence of the lymphoid cells concerned.

(4) Although the antigenicity of thyroid components apparently transgresses the law of self-tolerance, there is no real doubt about the " inaccessibility " of thyroglobulin, and probably of the microsomal C.F. antigen too, in relation to contact with antibody-producing tissues. Experimental findings indicate that such " inaccessible " antigens are also present in the central and peripheral nervous systems, the lens, the uveal tissue, the adrenal and the testis as well as in the thyroid. None of these tissues is subject to the scavenging of effete cells by macrophages.

In terms of Burnet's clonal selection theory of antibody production (Burnet, 1959*a*, 1959*b*), the existence of circulating antibody implies for an " inaccessible " antigen very much what it does for a foreign antigen. Entry of the antigen into " unshielded " tissues activates any corresponding clones of lymphoid cells present, which either have persisted from embryonic life or have arisen as a result of subsequent mutation. This activation and proliferation of preexisting clones will in the first place result in a delayed hypersensitivity-type of reactivity, and then a secondary stimulation will cause antibody production. Pathogenic activity of I.C.C. or circulating antibody will depend upon the possibility of a local vicious circle developing, and here we become involved in the effectiveness of homœostatic control in the individual, as well as accidental initiating factors such as infection and trauma. Normal homœostatic control is visualized, at least in part, as inhibition of autoantibody production by excess of antigen.

We must assume that under many functional stresses small amounts of thyroid antigens leak into the circulation. These will provoke only a trivial increase in the number of corresponding immunologically competent cells, without either antibody or tissue damage necessarily resulting; but this does provide a background of available reactivity. If a more effective leak of thyroid antigens occurs in an individual having good homœostasis, antibody production may be the only response; but in a person predisposed

to immunopathy we picture the sequence as follows.

With proliferation of I.C.C., these cells are attracted to the damaged region—tropism—where they will be in potential contact with antigen. The reactions resulting will be (*a*) of homograft type involving liberation of pharmacologically active material probably damaging both to I.C.C. and thyroid, and (*b*) proliferation *in situ* with production of plasma cells. This is likely to occur only if there is abnormal weakness of the homœostatic control (inhibition by excess of antigen). Our observations on the coexistence of thyroiditis with other systemic manifestations, notably rheumatoid arthritis and chronic hepatitis, may be cited as evidence for a more general failure of homœostatic control. A further point made by Burnet is that circulating antibody can under some circumstances reduce the potential pathogenicity of I.C.C., perhaps by removing the possibility of directive tropism. If " colonization " of the gland does not occur, this is likely to be the situation. This may be the explanation of high titres of circulating antibody unassociated with tissue damage.

The existence of circulating antibodies to antigens, such as thyroglobulin and the nuclear and cytoplasmic components of cells, provides suggestive evidence, albeit circumstantial, for the role of immune mechanisms in lymphadenoid thyroiditis, lupus erythematosus and other autoclastic diseases. However, it is uncertain whether circulating antibody ever initiates or perpetuates thyroiditis or other visceral disease in the human subject, and if so under what circumstances. Close consideration is being given to the hypothesis that " immunologically competent cells ", which mediate the delayed type of hypersensitivity, are more particularly implicated than circulating antibody. To find a method of demonstrating this " immunological competence " of antibody-producing cells constitutes the present challenge to the biologist.

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COMPLEMENT FIXATION WITH EXTRACT OF NORMAL THYROID GLAND: ITS OCCURRENCE IN SERA FROM PATIENTS WITH THYROID DISEASE AND IN OTHER CONDITIONS[1]

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SUMMARY

Attention is drawn to a complement-fixing agent which reacts with extract of normal thyroid gland. There are reasons for regarding this agent as an antibody, and possibly as an auto-antibody. It is significantly associated with thyroid disease, though not so closely as the complement-fixing anti-thyrotoxic antibody, or the anti-thyroglobulin antibody which reacts in the tanned-cell hæmagglutination technique. It is also associated with some other types of disease, particularly syphilis, the " collagen disease " group, and viral infections. In non-thyroid diseases, positive reactors are almost equally distributed between the sexes.

Any attempted synthesis of the current knowledge of the association between thyroid disease and the presence of serum auto-antibodies should take this agent into account, even though its activity or significance *in vivo* may be not entirely confined to the thyroid gland and its functions.

AN antibody which fixed complement with simple saline extracts of various organs including normal thyroid gland was found by Goudie *et alii* (1959) in two sera, which were not from patients with overt thyroid disease. These sera also fixed complement with thyrotoxic thyroid extract. Roitt and Doniach (1958) found that 13% of 167 sera from patients with thyroid disease which fixed complement with thyrotoxic thyroid extract reacted variously with brain, liver, kidney or suprarenal. They showed that the reaction, while not organ-specific, seemed to be related in some way to " auto-immune " thyroiditis. Gajdusek (1957, 1958) and Mackay and Gajdusek (1958) have reported the occurrence of this type of complement-fixation reaction with various tissue preparations in a number of diseases, particularly hepatitis, disseminated lupus erythematosus and macroglobulinæmia.

We have found sera which fixed complement with extract of normal thyroid gland in 24% of a series of 66 cases of thyroid disease, and in 11% of 945 mixed conditions not associated with thyroid signs or symptoms.

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MATERIALS AND METHODS

Sera were stored at −20° C. and inactivated for 30 minutes at 56° C. before being tested. The abbreviations N.T. and T.T. have been used in this report for extracts of normal thyroid gland and thyrotoxic thyroid gland respectively.

Source of Sera

Thyroid Disease.—Blood was taken in the Royal Adelaide Hospital in 66 cases in which a firm diagnosis of thyroid disease had been reached by the usual clinical and laboratory methods. The same cases, with three others, have been used as a control series in another study (Hackett *et alii*, 1960).

Non-Thyroid Conditions.—Blood was taken as a routine procedure from patients without thyroid disease admitted to the beds of the Medical Professorial Unit of the Royal Adelaide Hospital. Other sera were obtained from time to time from large groups of patients in other medical clinics of the hospital, and a few sera were specifically taken from patients suffering from syphilis, yaws, hepatic cirrhosis or " diffuse collagen disease ", or from patients known to have abnormal serum protein electrophoretic patterns. Each case was reviewed to confirm that signs or symptoms of thyroid disease were absent.

Complement-Fixation Test

Complement.—Pooled guinea-pig serum was stored in small amounts at −20° C.

Antigens.—Fresh organs obtained surgically or at autopsy were homogenized to a 10% suspension in physiological saline in a Waring blender, then centrifuged three times at 3000 r.p.m. for 10 minutes at +4° C. The sediments were discarded. The supernatant antigen was stored in small containers at —20° C. for up to three months. Antigens were prepared from normal thyroid gland, liver, kidney, lung and adrenal gland. Several thyroid glands or adrenals were pooled when making antigen from these organs.

Diluent.—Calcium magnesium saline was used throughout the test (Mayer *et alii*, 1946).

Technique.—A five-volume technique, using 0·2 ml. volumes in Wassermann tubes, was employed. Serum dilutions, antigen, and 2·5 HD_{50} of complement (titrated previously in the presence of antigen) were allowed to stand overnight at +4° C. and then for one hour at bench temperature. Two volumes of hæmolytic system were then added to each tube and incubated at 37° C. for 30 minutes, after which the cells were sedimented by light centrifuging; 50% hæmolysis was taken as the end-point. Each test was accompanied by a serum anti-complementary control, and known "positive" and "negative" sera were included in each batch of tests. Sera were initially screened at 1/10 and 1/80, and then titrated fully if complement fixation occurred. A positive result was complement fixation at 1/10 or a higher dilution.

Tanned-Cell Hæmagglutination Test

Boyden's technique (Boyden, 1951), using washed sheep-erythrocytes, was followed with the modifications introduced by Fisher (1952) and applied to the detection of antibodies specifically reacting with human thyroglobulin. Sera were absorbed with sheep cells before the test. An antigen-inhibition control, a diluent control, an untanned-cell control, an uncoated tanned-cell control, and known "positive" and "negative" serum controls were included in each run. Thyroglobulin was prepared by the method of Derrien *et alii* (1948). "Perspex" well-plates were used, and sera were put up in doubling dilutions starting at 1/10. A positive result was hæmagglutination at 1/10 or higher.

RESULTS

Sera from 1011 thyroid and non-thyroid hospital patients and from 164 healthy blood donors were tested for complement fixation with N.T. (Table I). The 123 positive reactors from this group were tested with T.T., and all these sera fixed complement.

Sera from 66 thyroid patients were tested for anti-thyroglobulin antibodies by the tanned-cell hæmagglutination method, as well as for complement fixation with N.T. The relationships between the results obtained by the two tests are shown in Table II.

Certain thyroid glands removed from thyrotoxic patients did not yield any T.T. antigen, and we had difficulty in maintaining a supply. (Goudie *et alii*, 1959, report that only one gland in five was suitable.) This shortage of antigen led to 25 of the 66 thyroid patients not being tested for complement fixation with T.T.; but the remaining 41 were tested for complement fixation with N.T. and T.T., as well as for the presence of anti-thyroglobulin antibodies by the tanned-cell hæmagglutination technique. The relationships between the results of these three tests on the thyroid patients are shown in Table III. It can be seen that 13 sera which fixed complement with T.T. did not do so with N.T.

TABLE I

Complement-Fixation with Normal Thyroid (N.T.) Antigen

Disease Category	Number of Tests	Positive Results	
		Number	Percentage
1. Thyroid diseases	66	16	24
2. Syphilis, yaws (positive Wassermann reaction)	15	11	73
3. Acute bacterial infections	68 } 79	1 } 2	3
Tuberculosis	11	1	
4. Viral infections	53	11	21
5. Diabetes	21	2	
Peripheral vascular diseases	8	0	
Arteriosclerosis, atheroma and sequelæ	80 } 142	5 } 11	8
Peptic ulcer and gastro-intestinal hæmorrhage	33	4	
6. Carcinoma, sarcoma	176	10	6
7. Reticuloses	37	1	
Lymphatic leukæmia	10	0	
Myelogenous leukæmia	10 } 84	0 } 4	5
Myeloproliferative diseases	15	0	
Paraproteinæmia dysproteinæmia	12	3	
8. Disseminated lupus erythematosus	13	9	
Idiopathic acquired hæmolytic anæmia	6 } 83	2 } 22	27
Rheumatoid arthritis	31	1	
Rheumatic fever	18	1	
Other "diffuse collagen" diseases	15	9	
9. Cirrhosis	60 } 78	8 } 12	15
Acute hepatocellular disease	18	4	
10. Glomerulonephritis	15	2	
Asthma	19	1	
Hypersensitivity, allergy	5	0	
Pernicious anæmia and hypochromic anæmia	19 } 235	1 } 21	9
Addison's disease	2	0	
Nephrotic syndrome	6	1	
Miscellaneous and undiagnosed	169	16	
Total	1011	120	12
Healthy blood donors	164	3	2

Complement fixation with N.T. was not confined to certain thyroid preparations. Sera reacting with N.T. did so consistently with all extracts made from different batches of pooled normal thyroid material.

With two exceptions (two cases of thyroid disease), every serum which reacted with N.T.

also fixed complement with one or more members of a range of similar saline extracts of human liver, kidney, adrenal or lung. The two exceptions gave weak reactions (1 : 10) with N.T., and they did not react with thyroglobulin in the tanned-cell test. The higher the titre of a serum with N.T., the more likely was it to react with all of the other four organ extracts rather than with only one or two, though this was not always so. Kidney and liver extracts fixed complement with various sera more frequently than adrenal and lung. Complement fixation titres with N.T. were not higher in sera from patients with thyroid disease than in non-thyroid conditions.

TABLE II

Sera from Patients with Thyroid Disease Tested by Two Tests

Thyroid Disease	Number Tested	Positive Results		
		Complement Fixation Test with N.T. Extract	Tanned-Cell Hæmagglutination Test for Thyroglobulin	Both Tests
Hashimoto's disease .. .. Primary myxœdema ..	26	7	19	
Thyrotoxicosis ..	23	6	13	4
Euthyroid goitre	15	3	9	2
Carcinoma of the thyroid ..	2	0	1	0
Total ..	66	16	42	13

Seventy-two of the 104 non-thyroid patients (Table I) whose sera fixed complement with N.T. were tested for thyroglobulin antibodies by the tanned-cell technique. Only 14 gave a positive result, and this proportion (19·5%) is similar to that found (17·6%) in 387 mixed non-thyroid patients not selected for complement fixation with N.T. and discussed by us elsewhere (Hackett *et alii*, 1960). We tested for thyroglobulin complement-fixing antibodies, and found none, in seven patients with Hashimoto's disease whose sera fixed complement with N.T., and whose serum also showed high-titre thyroglobulin antibodies by the tanned-cell technique. We have concluded that in most cases our N.T. complement-fixing antibody is not reacting with thyroglobulin (Anderson *et alii*, 1959).

DISCUSSION

The " thyrotoxic factor " is sometimes present in small amounts in normal thyroid glands (Roitt and Doniach, 1958). Therefore, it is possible that T.T. and N.T. are the same, differing only quantitatively. If this was so, one would expect to be able to titrate the T.T. antigen to very high dilutions with all sera which react with N.T., but this is not so. Further, if the two were the same, the activity of N.T.-reacting sera with other tissue antigens would have to be explained either on an assumption that thyrotoxic factor was to be found in organs other than the thyroid gland, or that the development of antibodies acting with traces of T.T. substance in normal glands was always, or nearly always, accompanied by the appearance of other anti-organ antibodies. This last would be curious, because many sera reacting with

TABLE III

Sera from Patients with Thyroid Disease Tested by Three Tests

Thyroid Disease	Number Tested	Positive Results							
		Complement-Fixation Test		Tanned-Cell Hæmagglutination Test for Thyroglobulin : C	A Only	A+C	A+B	B+C	A+B+C
		N.T. Extract : A	T.T. Extract : B						
Hashimoto's disease Primary myxœdema	10	4	5	8	0	4	4	5	4
Thyrotoxicosis..	16	5	12	10	0	3	5	7	3
Euthyroid goitre	13	3	7	8	2	2	3	4	2
Carcinoma of the thyroid ..	2	0	1	1	—	—	—	0	—
Total ..	41	12	25	27	2	9	12	16	9

TABLE IV

Complement Fixation (N.T.) Table Showing Total Distribution of Positive Results between Male and Female Patients, and the Effect of Thyroid Disease and Disseminated Lupus Erythematosus Upon It

Subjects	Males			Females		
	Total Number	Positive Results	Percentage	Total Number	Positive Results	Percentage
All subjects	466	45	9·7	545	75	13·8
Thyroid disease only	3	0	—	63	16	25·4
Disseminated lupus erythematosus only	1	0	—	12	9	75·0
All patients, less those with thyroid disease	463	45	9·7	482	59	12·2
All patients, less those with thyroid disease and disseminated lupus erythematosus	462	45	9·7	470	50	10·6

T.T. from thyrotoxic glands do not react with extracts of other organs. Only satisfactory absorption experiments could demonstrate a clear difference between N.T. and T.T., but it seems likely that it exists.

On the assumption that the two are different, the practical implication is that all sera reacting with N.T. will also react with T.T., because thyrotoxic glands always contain N.T. Unless the N.T. can be removed, which at present is not possible, complement fixation tests with T.T. should be controlled by testing simultaneously with other organ extracts, such as kidney, liver and lung. Only if reactions are not obtained with these can the result be regarded as reliable so far as the detection of "thyrotoxic" antibody is concerned.

The results of N.T. complement fixation testing and the patients' diseases in 945 non-thyroid cases (together with the total results in thyroid disease) are given in Table I. The allocations of individual cases to disease classifications were made after a full review of each case and before the serological results were known. The diseases are divided into 10 groups, the associations within the groups being apparent in most of them. Group 5 is one of the loosest, and comprises disorders of the arterial tree in a wide sense, including infarction and its sequelæ, and hæmorrhage, but not periarteritis nodosa, which is in Group 8. Group 10 is heterogeneous, being made up of the residue, together with undiagnosed conditions.

It is clear that certain of the groups, and the total sample, show a higher incidence of N.T. antibody in the serum than is found in healthy people represented by 164 blood donors. However, we wished to make comparisons among different disease states rather than between disease and health. We decided that Group 10 represented a satisfactory sample of mixed hospital cases from the same wards, as those which could be clearly separated into distinct disease categories of similar size. Consequently we have compared the rate of positive reactions in the groups in turn with Group 10, using the χ^2 test. Groups 1, 2 and 8 all show very significant differences from Group 10 ($P = <0{\cdot}002$, $<0{\cdot}001$ and $<0{\cdot}001$ respectively), while Group 4 is less significantly different ($P = <0{\cdot}05$).

None of these N.T. reactions bore any direct or reciprocal relationship to any of the frequently occurring blood-group antigens, to recent blood transfusion of the patients, to positive results with the Rose-Waaler or Paul-Bunnell tests, or to the presence of C-reactive protein, all of which were checked on sections of the data. A comprehensive check was made on 44 "positive" N.T. sera to see whether their activity might be in part due to complement fixation by an anti-A blood-group antibody reacting with A-substance in the antigen. No evidence of such activity was found.

The sex incidence of N.T.-positive reactors among the non-thyroid subjects was 9·7% of men and 12·2% of women. Table IV shows the effect on this of removing 13 cases of disseminated lupus erythematosus, which was to reduce the sex difference to give an incidence of 9·7% in men and 10·6% in women. An analysis by decades of the ages of the non-thyroid subjects showed no relationship of positive N.T. results to any age group, and no rising incidence with increasing age in either sex.

Correlation of the N.T. Complement-Fixing Antibody with Disease

The N.T. antibody is significantly associated with thyroid disease, but not so strongly as the T.T. antibody or the thyroglobulin-reacting antibody detected by the tanned-cell hæmagglutination test. The incidence of these three antibodies in our cases of thyroid disease is respectively 24%, 61% and 67% (Tables II and III).

In non-thyroid disease, the presence of the N.T. antibody is significantly associated with syphilis and yaws (73%), with " diffuse collagen disease " (27%) and with viral infections (21%). In the total of 945 mixed medical teaching hospital patients unclassified except for their confirmed lack of thyroid signs and symptoms, there are 104 positive reactors (11%—Table I), so it seems unlikely that the N.T. antibody damages the thyroid. If it does, it is not to a point where the damage is recognizable as clinical thyroid disease.

Thyroid disease has a marked female sex incidence, and the general distribution of the N.T. antibody in our 945 non-thyroid people has only a slight female predominance, while an analysis by decades of age does not show any rising incidence in older people of either sex. This contrasts with the predominant incidence of anti-thyrotoxic antibody among older females (Goudie *et alii*, 1959), and of anti-thyroglobulin antibody (Hackett *et alii*, 1960) in hospital patients who have no clinically apparent thyroid disease, but who may have occult thyroiditis or benign lymphocytic infiltration of the gland.

If there is a link between the groups of diseases (including the thyroid disorders) which give the highest proportion of positive N.T. results in Table I, it is not obvious. One might postulate that a common factor was lymphocyte or plasma-cell hyperplasia somewhere in the body. The appearance of the "auto-antibody" might then be the result of the growth of a rogue clone of " forbidden " cells on Burnet's hypothesis (Burnet, 1959). In the latter case, its notable absence in our patients with reticulosis and leukæmia could be due to the mesenchymal niches in such conditions being wholly occupied by a single neoplastic clone without anti-thyroid configurations. Competition from such a growth could prevent the development of a variety of hyperplastic cell lines (including various " forbidden " clones), to which there may be a tendency in diseases involving a non-neoplastic lymphocytosis or " small round cell infiltration " of the tissues.

The Status of the N.T. Complement-Fixing Antibody

Roitt and Doniach (1958) discuss the question of whether this serum reaction involves an orthodox antibody-antigen reaction. They refer to Cruickshank (1958), who found that some sera which fixed complement with a tryptic digest of kidney could not be absorbed by a wet preparation of glomeruli, and that the complement-fixing activity was not confined to the gamma-globulin fraction of the serum. Absorption experiments using tissue extracts or homogenates, or fresh organ slices, are in our experience very difficult to carry through successfully with a complement-fixing antibody, owing to the ease with which anti-complementary substances or additional soluble antigen can be released into the serum during the experiments.

According to Burnet (1959), " Any population of globulin molecules in a body fluid which can unite to a specifically limited range of chemical patterns can justifiably be called antibody ". Talmage (1957) states that " The only properties common to all antibodies are (*a*) their protein nature, (*b*) an increased production following exposure to antigen, and (*c*) affinity for the antigen ". The agent we are dealing with here satisfies (*a*) and (*c*), while (*b*) has not yet been determined.

We believe that in this N.T. complement-fixation reaction we have the phenomenon described by Gajdusek (1957, 1958) and by Mackay and Gajdusek (1958) as " auto-immune complement-fixation ", which they found particularly in cases of hepatic disease, collagen disease and paraproteinæmia. Complement was fixed with homogenates of various organs, including thyroid gland. Gajdusek (1958) showed that all the activity lay in the gamma-globulin fraction of serum, and we accept his reasons for regarding the reacting agent as a genuine antibody. It gives as definite and as consistent complement fixation as does the antibody (or reagin) responsible for a positive Wassermann reaction, the prototype of this kind of test.

We hold the view that those who seek to synthesize current concepts of an auto-immune component in thyroid disease should take the N.T. antibody into account. Its antigen (or substance with which it unites specifically) is certainly in the thyroid, wherever else it may occur in the body.

ACKNOWLEDGEMENTS

We wish to thank Professor H. N. Robson and other consultants of the Royal Adelaide Hospital for allowing us access to their patients, Dr. Kathleen Maros for help with the sorting and recording of case histories, and Miss N. Deering and Miss B. Rainsford for valuable technical assistance.

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PRECIPITIN TESTS FOR AUTO-IMMUNIZING THYROIDITIS[1]

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SUMMARY

The results of precipitin and flocculation tests on the serum of 76 patients with thyroid disease are presented and discussed. A positive precipitin reaction to thyroid extract indicates that, at one time or other, the patient has become sensitized to thyroglobulin.

A thyroid precipitin test employing normal electrophoretic equipment is described. The test is slightly more sensitive, simpler and more rapid than the agar diffusion test. Taken in conjunction with clinical assessment and other available investigations, this precipitin test is a valuable diagnostic procedure.

DURING the past six years, great advances have been made in laboratory aids to the diagnosis of Hashimoto's disease (struma lymphomatosa, lymphadenoid goitre). In this condition, the results of empirical turbidity and flocculation tests—the so-called liver function tests—were first observed by Fromm, Lascano, Burr and Escalante (1953) to be often abnormal. This finding, confirmed by Luxton and Cooke (1956), and by Skillern, Crile, McCullagh, Hazard, Lewis and Brown (1956), led Roitt, Doniach, Campbell and Hudson (1956) to postulate and prove that lymphocytic infiltration of the thyroid together with an excess of circulating serum globulins was evidence of an immune reaction. At the same time, the development of tracer techniques with radioiodine, in particular the ^{131}I perchlorate test of Morgan and Trotter (1957), has demonstrated that there is a delay in the binding of iodine to thyroid protein in auto-immunizing thyroiditis.

Three tests have been developed to demonstrate the presence of thyroid antibodies in serum — an agar-diffusion precipitin test (Doniach and Roitt, 1957), a complement fixation test and a tanned-red-cell agglutination test (Roitt and Doniach 1958)—and it is now clear that positive results with these tests are indicative of a far-ranging immune response to thyroid proteins which, for reasons not always apparent, have leaked from their normal site. The complement fixation test, considered by Witebsky (1957) to be the least satisfactory of all tests for lymphadenoid goitre, has been shown in several laboratories to give up to 9% positive results in women with normal thyroid function. The tanned-red-cell agglutination test, an adaptation of the method of Boyden (1951), is an extremely sensitive test, giving positive results in most cases of spontaneous myxœdema, and in some cases in women with no known disease of the thyroid (Owen and Smart, 1958). On the other hand, while the much less sensitive agar-diffusion precipitin test gives no false positive results with serum from normal people, several reports of false negative observations in Hashimoto's disease, varying from 25% to 50%, have appeared in the literature.

However, it is our experience that a good correlation exists between the results of the agar-diffusion test and the presence or absence of untreated lymphadenoid goitre. Furthermore, the need for a convenient screening test for this disease appears to have been fulfilled by a simple technique previously outlined (Watson and Whinfrey, 1958). Therefore, the purpose of this communication is to describe in detail these precipitin tests, and to present the results obtained by them in a number of female hospital patients.

METHODS

The " liver function " tests used in this study were (*a*) the thymol turbidity test (Maclagan, 1954), (*b*) the zinc sulphate turbidity test (Kunkel, 1947), and (*c*) the colloidal gold reaction (Maclagan, 1946) Determinations of serum alpha-2, beta and gamma globulins were made by eluting the protein-bound dye from the relevant portions of the filter-paper strip used in the electroprecipitin test for thyroid antibodies (Varley, 1958). An estimate of the individual globulins was obtained from the total protein figure (found by a copper sulphate specific gravity method) and from the percentage of dye bound by each globulin.

[1] Received on November 13, 1959.

[2] Chief Biochemist.

Electroprecipitin Test for Thyroid Antibodies in Serum

The following materials are required: (i) patients' serum free from hæmolysis; (ii) stock thyroid extract; (iii) dilute antigen; (iv) barbitone buffer. The stock thyroid extract is prepared as follows:

Portions of three or four thyroid glands are collected. Post-operative or fresh post-mortem specimens are suitable, provided that the tissue shows some colloid within the lumen of the follicles. Each is washed well under running water to free it from excess blood, and is then stored at —20° C. until required. By means of a razor, very thin shavings are removed from each of the glands, and these are soaked overnight at

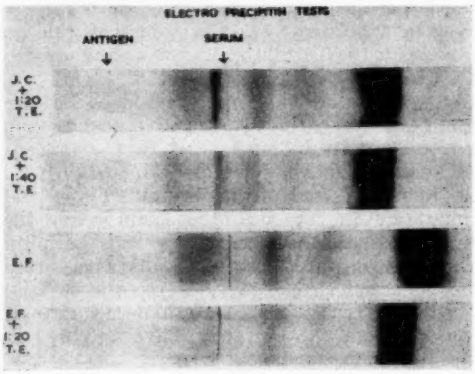

FIGURE I

Illustrating precipitin patterms. Serum from patient J.C., positive result with thyroid extract 1 in 20 and 1 in 40; serum from patient E.F. control, and positive result with thyroid extract 1 in 20

2° to 4° C. in an equal volume of 0·9% sodium chloride solution. The mixture is then centrifuged for 10 minutes at 3000 r.p.m., and the supernatant extract is collected and divided into 1 ml. portions and stored at —20° C. When this stock extract is diluted 1 in 20, the solution should be pale straw-coloured, and contain insufficient albumins and hæmatins to be detectable by electrophoresis as described below.

The dilute antigen is prepared by diluting the stock thyroid extract 1 : 20 with 0·9% sodium chloride solution and storing it in the refrigerator. This should be well mixed before use, and discarded immediately a slight precipitate appears.

The barbitone buffer has a pH of 8·6 and an ionic strength of 0·06 to 0·08.

Electrophoresis equipment is set up in the normal manner, with a strip of filter-paper Whatman No. 3MM (" special for chromatography "), 5 cms. wide. An amount of 0·05 ml. of the patient's serum is applied in the usual place, and 0·05 ml. of the dilute antigen is applied 4–5 cm. behind the serum (see Figure I). The exact spacing of these fluids and the length of time for which the electromotive force should be applied will to some extent depend on the make of equipment employed. The antigen contains thyroglobulins which run with the speed of an alpha-1 globulin, and it is necessary for this solution to have completely traversed the beta-globulin of the serum by the time when the serum albumin and individual globulins have become separated.

After separation, the papers are dried thoroughly at 105° C. and stained with bromphenol blue in the usual way. A deeply-stained sharp line or diffuse band situated within or adjacent to the serum gamma-globulin fraction denotes the presence of thyroglobulin antibodies in the original serum (see Figure I). A rough estimate of the antibody titre can be made by repeating the test using more dilute antigen. During this study, the lowest concentration giving a positive result was a 1 : 200 dilution of antigen.

With the use of an extract of pooled thyroid glands as described above, very little variation in antigen concentration has been encountered. However, once an antibody-containing serum has been found and the strength of the reaction observed, the antigen strength of an extract from a fresh batch of glands can be adjusted with the aid of a secondary standard of pure human thyroglobulin. This is prepared by the method of Derrien, Michel and Roche (1948). Under the conditions of the test, a 1% solution of thyroglobulin (obtained by shaking the crystals vigorously in 0·9% sodium chloride solution) has about the same degree of reactivity towards serum antibodies as that of the 1 : 20 dilute antigen.

The Agar Precipitin Test

This method is essentially that described by Doniach and Roitt (1957); a few modifications have been introduced. The stock antigen is prepared as described above, and for use this is diluted five times with 0·9% sodium chloride solution. New Zealand agar (1%) is cleared with the aid of 1·5% w/v of equal parts of the clarifying agents bentonite and " Hyflo-Super-Cel " (Feinberg, 1956). This facilitates the detection of weak precipitin reactions. Sodium chloride solution (0·9%) and sodium azide (1%—preservative) are added. The procedure adopted is as follows.

The agar is melted and kept at 40° to 45° C. With Pasteur pipettes, several drops of the patient's serum are added and mixed with an equal volume of the agar. This is then transferred to the bottom of a small tube (75 mm. by 7 mm.). When the mixture has set, agar diluted with an equal volume of 0·9% sodium chloride solution is carefully layered above it, to 1 cm. in depth, and allowed to set. Finally, some 1 : 5 antigen is added and mixed with an equal volume of agar in another tube and superimposed on the other. The tubes are stored at 2° to 4° C., and examined every 48 hours for the presence of a thin white line in the centre of the middle layer. Up to 10 days may often be required before a positive reaction becomes apparent.

It is essential to avoid air bubbles in any of the agar mixtures, and care must be taken not to touch the side of the tube when introducing or removing the Pasteur pipette.

RESULTS AND DISCUSSION

Precipitin tests were carried out on 188 specimens of serum from 129 patients. These were 83 patients presenting with suspected thyroid disease, 30 patients not regarded as having thyroid disease and 16 healthy women. The results of these tests are summarized in Table I.

TABLE I

Results of Precipitin Tests in Hospital Patients with Various Conditions

Diagnosis	Number of Subjects Tested	Number of Positive Results
Myxœdema	11	2
Hashimoto's disease	25	24
Non-toxic goitre	8	0
Toxic nodular goitre	11	0
Thyrotoxicosis	13	0
Thyroid cancer	3	1
Other thyroid disease	5	0
"Non-thyroid" disease	30	0
No abnormality (female subjects)	16	0

Of 25 patients subsequently diagnosed as having auto-immunizing thyroiditis, 24 gave a precipitin reaction against thyroid extract. Thyroglobulin agglutinins were detected in the other patient's serum by means of the more sensitive tanned-red-cell test. Serum from five of these patients was examined only by the agar-diffusion method. Tests in the other 19 cases were made on at least one occasion by both the agar and the electrophoretic techniques, and in most instances were made before, and at approximately monthly intervals during, treatment with thyroid or *l*-thyroxine. The prevalence of positive precipitin reactions with the serum from these patients is set out in Table II.

TABLE II

Results of Thyroid Precipitin Tests Made at Approximately Monthly Intervals in 19 Cases of Hashimoto's Disease

Number of Cases	Month						
	0	1	2	3	4	5	6
3	+	+	−				
4	+	−	−				
2	+	+	−	+	−	−	
2	+	+	+	+			+
5	+						
1	−	−	+	−	−	−	
1	−	−	−	−	+	−	−
1	+		+				

It will be seen that the serum of two patients who initially showed a positive reaction later gave a negative result, then a positive result and finally a negative result. Also, the serum of two other patients gave no precipitin reaction—one up to two months, one up to four months—before a positive result was observed. Each of these later positive reactions was obtained by the electroprecipitin test; the agar-diffusion tests gave negative results, except that with one serum a precipitin reaction was detected when the antigen was further diluted to 1 : 20. It is concluded that the electroprecipitin method is slightly more sensitive than the agar-double-diffusion method, although there is no doubt that in many sera the antibody when present is close to the limiting level of the sensitivity of the reaction. In other laboratories, the results of precipitin tests on four series of patients with untreated Hashimoto's disease are shown in Table III. In the present series a higher proportion of positive results was obtained than has been reported by other workers. A few of the "positive" sera were tested against extracts of pig and sheep thyroid and against extracts of human organs other than thyroid gland; in no case was a precipitin reaction observed.

TABLE III

Percentage of Positive Results to Precipitin Tests in Untreated Hashimoto's Disease

Author	Number of Subjects Tested	Percentage of Positive Results
Trotter *et alii* (1957)	24	50
Goudie *et alii* (1957)	12	75
Roitt *et alii* (1958)	106	70
Doniach *et alii* (1958)	44	66
Present study	25	96

The demonstration of a serum-immune reaction to thyroid extract does not necessarily indicate that an active process is interfering with the thyroid cells, but simply that the patient must have become sensitized to thyroglobulin at some time or other. In two patients who gave positive responses to precipitin tests over a period of one and three years respectively after thyroid therapy was instituted, the persistent immune reaction was accompanied by the presence of greatly increased serum gamma globulin. In the second of these cases, the serum gamma globulin level fell slowly from 2·73 to 2·00 grammes per 100 ml. over the three-year period. An advantage of the electroprecipitin test is that it permits the simultaneous assessment of serum albumin and globulin fractions. In this study three flocculation tests were also carried out. Contrary to

TABLE IV

Results of Tests for Abnormal Globulins in 43 Cases of Thyroid Disease

Condition	Number of Subjects with Abnormal Proteins (Electrophoresis)		Number of Subjects with Positive Findings in Flocculation Tests		
	Beta Globulin (>1·0 gramme per 100 ml.)	Gamma Globulin (>1·6 grammes 100 ml.)	Colloidal Gold (>1+)	Thymol Turbidity (>4 units)	Zinc Sulphate (>9 units)
Untreated: Hashimoto's disease (20 patients)	6	9	7	8	8
Other: thyroid disease (23 patients)	3	6	6	4	7

the experience of Goudie *et alii* (1957), neither the serum flocculation tests nor the estimation of beta and gamma globulins (Table IV) proved to be as sensitive an index of thyroiditis as the precipitin test. Evidence of a serum protein abnormality, as shown electrophoretically or by at least one of the empirical tests, was obtained from 11 of 20 patients with Hashimoto's disease, and from eight of 23 patients with other thyroid disorders. No single flocculation test appeared to offer an advantage over the others as a diagnostic aid.

One of the three patients with thyroid cancer had thyroid antibodies in her serum; the electroprecipitin test gave a positive result, but unfortunately the agar test was not done. The patient, *post mortem*, was found by Dr. G. R. Osborn to have multiple lymphosarcomatous deposits, one of which occupied the region of the thyroid gland. This finding supports the view that a firm distinction between lymphadenoid goitre and thyroid cancer cannot be made by means of antibody tests.

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SMALL-BOWEL BIOPSY WITH THE SUCTION BIOPSY TUBE[1]

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SUMMARY

In 1948 a suction gastric biopsy tube was designed in our Unit. In 1956 Shiner, of London, modified this tube for jejunal biopsy. In the present study minor alterations were made to Shiner's tube, and 51 successful biopsies were performed on 46 patients at 74 attempts, fragments of jejunum being obtained in 38 and fragments of duodenum in 13.

Eight representative cases and their corresponding biopsy findings are described, the conditions present including atrophic gastritis, pernicious anæmia and subacute combined degeneration of the cord with gastric atrophy, malabsorption with steatorrhœa and Whipple's disease.

Pronounced jejunal atrophy was present in two cases of malabsorption, and the villi were distended with P.A.S.-staining cells in the case of Whipple's disease. No constant major abnormality of the jejunum was found to be associated with atrophic gastritis or gastric atrophy.

Our limited experience suggests that gastric atrophy and jejunal atrophy have not a common causal factor.

DURING 1948, the Clinical Research Unit of the Royal Melbourne Hospital and the Walter and Eliza Hall Institute was the first to develop the flexible suction gastric biopsy tube, and 55 successful gastric biopsies were described in *The Lancet* of January 1, 1949 (Wood *et alii*, 1949). Since that time further studies have been reported by members of the Unit, notably by Doig *et alii* (1950), by Motteram (1951), by Funder and Weiden (1952), and by Joske *et alii* (1955). In 1958 Wood and Taft gave the Unit's total experience with gastric biopsy; there had been 1949 attempts at biopsy and 89% had been successful.

In 1956 Dr. Margot Shiner, of the Postgraduate Medical School, London, modified our tube for the performance of duodenal and jejunal biopsies (Shiner, 1956*a*, 1956*b*; Doniach and Shiner, 1957).

In 1958 a symposium on disorders of the small intestine was held by the Royal Society of Medicine in London, when the value of Shiner's work was fully appreciated (Royal Society of Medicine Symposium, 1959). Further confirmation of her work came from Culver *et alii* (1959) in the United States of America. In 1958 one of us (I.J.W.) was privileged to see Cyrus Rubin's studies in the United States of America, and we are indebted to him for his guidance in the design of our small-bowel biopsy tube (Brandborg *et alii*, 1959).

CONSTRUCTION AND USE OF SMALL-BOWEL BIOPSY TUBE[1]

As shown in Figures I and II, our tube is 150 cm. long, and consists of a length of Bowden wire covered by plastic tubing, the overall diameter being 4 mm. At the proximal end there is a lateral tube through which negative pressure is applied. An operating wire passes through an airtight gland, down the length of the flexible tube, to operate a cylindrical knife in the operating head. Pushing the blade down by the operating wire exposes a lateral hole in the operating head, through which a fragment of mucosa can be sucked. The fragment is cut off by pulling the knife past the hole by means of the operating wire. The fragment is obtained by withdrawing the tube and unscrewing the operating head and cylindrical blade. Three marks—I, II and III—are placed 75, 100 and 125 cm. respectively from the operating head. When the tube is passed to the level of mark I, the tip will be at the level of the pylorus if it has taken the direct route.

[6] Presented at the inaugural meeting of the Gastroenterological Society of Australia in Adelaide in May, 1959. Received on October 15, 1959.

[2] Pathologist to the Clinical Research Unit.

[3] Head of the Clinical Research Unit.

[4] Engineer, Walter and Eliza Hall Institute.

[5] Working with the aid of a grant from the National Health and Medical Research Council of Australia.

[1] This biopsy tube is made by H. A. Taylor and Son (Surgical) Pty. Ltd., 44 Macfarlan Street, South Yarra, Melbourne, Victoria.

Passing the Tube into the Jejunum

With the patient fasting, the throat is anæsthetized and the biopsy tube is passed into the upper part of the œsophagus with the patient sitting. He then lies on his right side on the X-ray screening table, and the

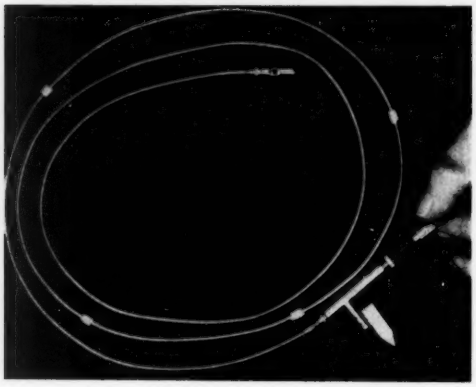

FIGURE I

Small-bowel biopsy tube with operating wire, lateral tube for suction (arrow) and operating head with lateral hole, through which a mucosal fragment is sucked and cut off with the cylindrical knife

tube is fed slowly down to mark I. Fluoroscopy then reveals the position of the operating head. It is usually pointing towards the pylorus, and by gentle abdominal manipulation can be coaxed into the duodenum and

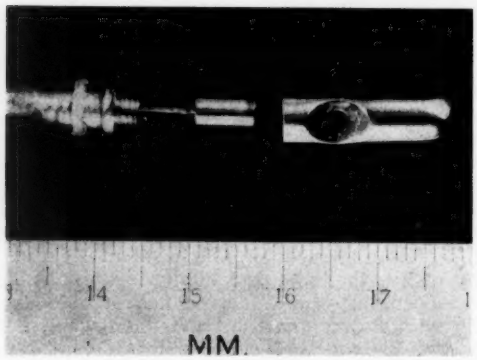

FIGURE II

Operating head dismantled to show cylindrical knife and operating head with lateral hole

then into the jejunum as far as the splenic area (Figure III). If the tube is arrested at the pylorus, the patient is returned to bed to rest on his right side for one hour. By this time the tube has usually passed into the duodenum.

Cutting the Fragment

When the tube reaches the splenic area, the biopsy fragment is cut with the patient lying on his right side to drain the bowel, or on his left side for duodenal biopsy. The knife blade is depressed by the operating wire, and negative pressure (20 in. of water) is applied for 5 sec. to suck in the fragment of mucosa. The fragment is cut off by firmly pulling up the knife blade. Usually two further cuts are made, the tube being withdrawn about 3 cm. after each cut. The fragments are gently spread on filter paper, fixed in formol saline, and immediately photographed under fluid to display the villi and contained blood vessels (Figure IV and V).

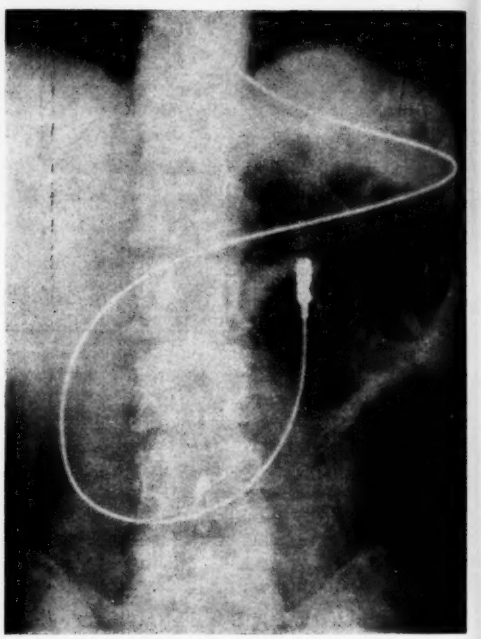

FIGURE III

X-ray view, showing biopsy tube passing through stomach and duodenum into upper part of jejunum

Efficiency of the Biopsy Technique

With this flexible tube, biopsies were attempted on one or more occasions on 59 subjects, and suitable fragments were obtained from 46. Our overall experience has been 74 attempted biopsies with 51 (69%) successes, the fragments being obtained from the jejunum in 38 and from the duodenum in 13. The failures were due to arrest of the biopsy tube at the pylorus in five cases, and failure to obtain a fragment after correct passage of the tube in 18. Our success rate was increased by the introduction of abdominal palpation and improvement in the structure of the tube.

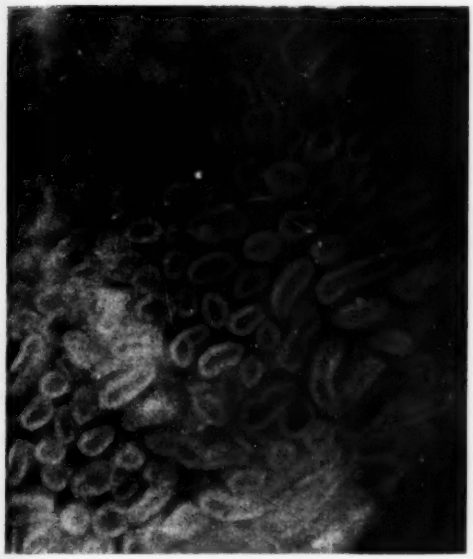

FIGURE IV

Normal jejunal mucosa. Surface view with well-formed villi photographed under fluid immediately after being obtained. (Unstained, ×25)

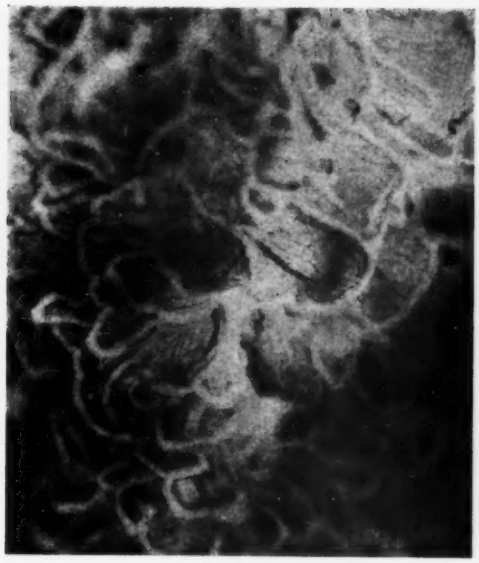

FIGURE V

Normal jejunal mucosa. Surface view similar to Figure IV. The leaf-like villi with capillary network are well shown. (Unstained, ×40)

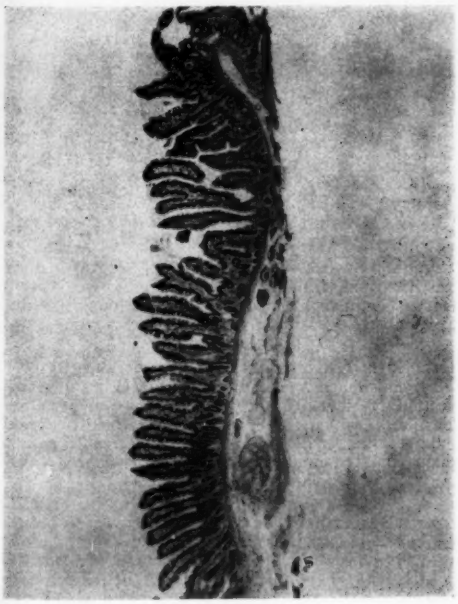

FIGURE VI

Normal jejunum. Section showing the extent of biopsy fragment. The villi are long and narrow. (Hæmatoxylin and eosin stain, ×24)

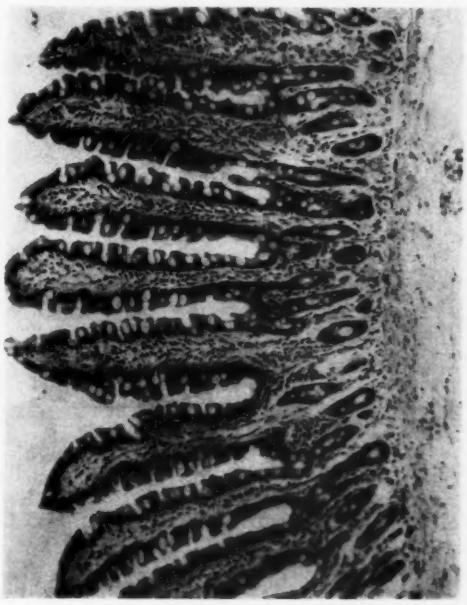

FIGURE VII

Normal jejunum. Similar view to Figure VI, showing well-formed intestinal villi with only scanty accumulation of cells in the lamina propria. (Hæmatoxylin and eosin stain, ×100)

HISTOLOGICAL FINDINGS

Normal Jejunal Mucosa

Figures IV and V give the surface view photographed at low magnification (×25) immediately after the specimen was obtained. The characteristic leaf-like form of the villi is readily seen, together with the capillary network within their substance. The shape of the villi vividly demonstrates the great expanse of absorbing surface available in the small bowel.

Examination of sections (Figures VI and VII) shows that the biopsy fragment comprises mucosa, muscularis mucosæ and often a trace of submucosa. In the mucosa there are projecting villi interspaced by crypts extending into the lamina propria. In the lamina propria there is a loose texture of connective tissue containing a variable number of lymphocytes and plasma cells.

Small-Bowel Mucosa in Disease

The clinical diagnosis in the 46 cases investigated and the corresponding findings at small-bowel biopsy are summarized in Table I. These findings are amplified in the following eight case reports with the findings at small-bowel biopsy. They are representative of our experience and indicate the value of this procedure.

TABLE I

The Findings in 51 Successful Biopsies Performed on 46 Patients

Clinical Diagnosis	Appearance of Mucosa on Section		
	Normal	Minor Abnormality	Pronounced Abnormality
Pernicious anæmia	12	0	0
Chronic gastritis	6	0	0
Peptic ulceration	3	1	0
Chronic alcoholism	4	1	0
Chronic pancreatitis	6	2	0
Carcinoma of pancreas ..	3	1	0
Malabsorption	6	1	2
Whipple's disease	1	0	1
Eosinophil granuloma of stomach	1	0	0

REPRESENTATIVE CASES

The first group of five cases was planned to discover whether gross diffuse changes in the gastric mucosa would be reflected in the small bowel. They are described in brief, as follows.

CASE I.—The clinical diagnosis was alcoholism with chronic atrophic gastritis and normal findings on jejunal biopsy. A labourer, aged 58 years, had suffered from alcoholism and malnutrition for the past 10 years. Gastric biopsy revealed moderately severe atrophic gastritis, with some atrophy of the acid and pepsinogen secreting cells and cellular infiltration throughout the lamina propria. However, the surface of the jejunal biopsy specimen showed a normal villous pattern, and the section was normal.

CASE II.—This was a case of severe atrophic gastritis; the jejunal biopsy findings were normal. A carpenter, aged 47 years, had suffered from intermittent flatulent dyspepsia, epigastric tenderness and achlorhydria for 23 years. During the last seven years repeated gastric biopsies all revealed severe atrophic gastritis, with heavy cellular infiltrate and great reduction of gastric glands and their specialized cells (Figure VIII). When last examined the patient was well and receiving no treatment. Examination of his peripheral blood and a bone marrow smear, and estimation of the serum vitamin B_{12} level, provided no evidence of pernicious anæmia. In contrast to the abnormal gastric biopsy findings, the small bowel biopsy revealed a normal villous pattern, and there was only a slight cellular infiltrate in the lamina propria.

FIGURE VIII

Case II. Gastric biopsy showing severe atrophic gastritis from a male, aged 47 years, with chronic dyspepsia and achlorhydria. Jejunal biopsy findings were normal. (Hæmatoxylin and eosin stain, ×100)

CASE III.—The diagnosis was pernicious anæmia and gastric atrophy; normal findings were obtained on jejunal biopsy. A male pensioner, aged 75 years, suffered from severe pernicious anæmia, the hæmoglobin value being 5·9 grammes per 100 ml., the red cell count 1,800,000 per cubic millimetre, and the M.C.V. 100 cubic μ; the bone marrow was megaloblastic, the serum vitamin B_{12} level was 25γγ per millilitre, and the Schilling test showed impaired absorption of vitamin B_{12}. He responded rapidly to vitamin B_{12} therapy. Gastric biopsy before this treatment revealed gastric

atrophy, typical of pernicious anæmia. There was almost complete loss of acid and pepsin-secreting cells and minimal cellular infiltrate. However, the jejunal biopsy specimen, obtained at the same time, was of normal appearance, both on inspection of the surface and on examination of sections.

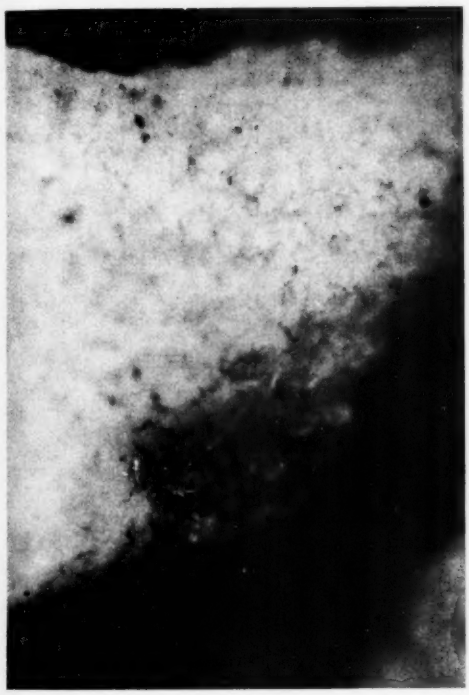

FIGURE IX

Case VI. Jejunal biopsy in intestinal malabsorption from a male, aged 53 years, with steatorrhœa and anæmia. Surface view showing absence of villi. (Unstained, ×25)

This has been our experience with pernicious anæmia, and it is that of Shiner (Royal Society of Medicine Symposium, 1959), who found normal intestinal mucosa in all of the eight cases.

CASE IV.—This was a case of prolonged gastric atrophy with ultimate development of pernicious anæmia and subacute combined degeneration of the cord. The jejunal biopsy specimen was normal. Ten years ago a miner, aged 65 years, reported to our Unit complaining of flatulent dyspepsia present for 40 years. He had diffuse epigastric tenderness, but X-ray examination with a barium meal revealed no abnormality. Gastric biopsy revealed pronounced gastric atrophy with extensive intestinal metaplasia, as previously reported by Robertson *et alii* (1955). Clinical examination and examination of the peripheral blood and a bone-marrow smear then showed no evidence of pernicious anæmia. However, five years later the patient first developed overt signs of classical pernicious anæmia and subacute combined degeneration of the cord, confirmed by the finding of a low serum vitamin B_{12} level. He was cured by vitamin B_{12} therapy. Recently a Schilling test by Dr. D. Cowling revealed deficient absorption of vitamin B_{12}. As would be expected, serial gastric biopsies over the 10 years during which he attended our Unit demonstrated that the gastric atrophy remained unchanged. A jejunal biopsy performed in 1959 produced a specimen within normal limits, both in its surface appearance and on examination of sections.

CASE V.—This was a case of post-gastrectomy pernicious anæmia, with normal jejunal biopsy findings. Ten years ago a cinema operator, aged 50 years, suffered from duodenal obstruction from an ulcer. A Pólya subtotal gastrectomy was performed. He remained well for three years, and then developed an anastomotic ulcer with perforation and hæmorrhage. A further gastrectomy was required, only a small portion of stomach being left. Nine years after the first subtotal gastrectomy he developed overt pernicious anæmia with typical changes in the peripheral blood, bone marrow and serum vitamin B_{12} level. He responded to vitamin B_{12} therapy. Biopsy of the stomach remnant revealed chronic gastritis with severe atrophy. The findings on jejunal biopsy were within normal limits.

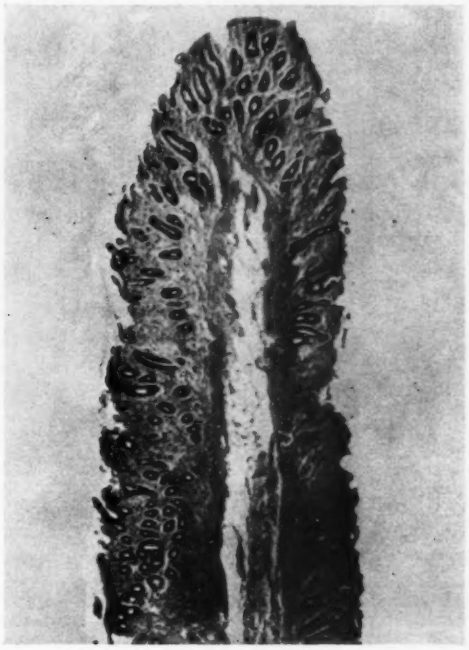

FIGURE X

Case VI. Jejunal biopsy in malabsorption. Examination of sections confirms gross atrophy with absence of villi. (Hæmatoxylin and eosin stain, ×100)

Cases I to V demonstrate that in our experience severe atrophic gastritis and gastric atrophy, with or without pernicious anæmia,

are not associated with pronounced changes in the jejunum. In contrast, three further cases are described, two of intestinal malabsorption and one of Whipple's disease.

CASE VI.—This was a case of malabsorption with steatorrhœa and anæmia. In 1950 Dr. R. Andrew referred to us a hotelkeeper, aged 53 years, who for 20 years had suffered bouts of diarrhœa with bulky

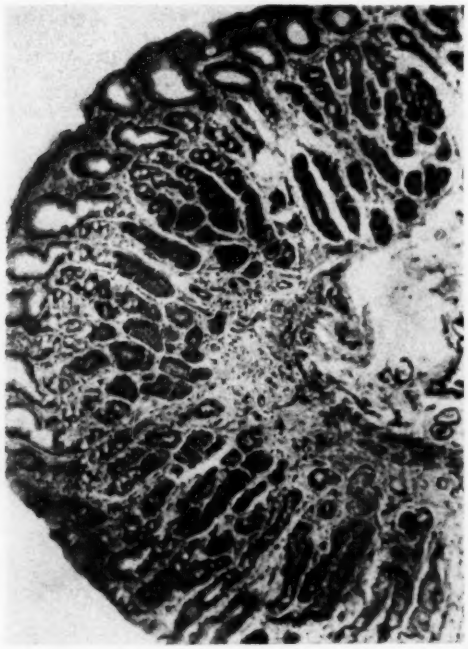

FIGURE XI

Case VI. Gastric biopsy in malabsorption. In contrast to the jejunal biopsy (Figure X), this mucosa shows normal structure. (Hæmatoxylin and eosin stain, ×100)

offensive stools. During the past four years these bouts had worsened, and he had suffered from anæmia and lost two stone in weight. Macrocytic anæmia and deficient intestinal absorption, especially of fat, were present. At the Repatriation General Hospital, Heidelberg, his condition was improved by treatment consisting of a diet of high protein and low fat content, vitamin B_{12}, folic acid and vitamin concentrates.

A jejunal biopsy specimen in 1959, when he was being controlled by treatment, presented an appearance strikingly different from normal. In the surface view there were no villi projecting from the surface (Figure IX). Examination of sections confirmed their absence (Figure X). In contrast, the gastric biopsy specimen was normal (Figure XI). In 1959 Shiner reported similar jejunal biopsy findings in seven cases, and in six of these the gastric biopsy specimen was normal.

CASE VII.—A female pensioner, aged 70 years, was a second patient suffering from malabsorption with steatorrhœa, anæmia and pronounced osteoporosis. A jejunal biopsy revealed similar findings to those in Case VI, there being pronounced atrophy of the villi (Figure XII). Again, the gastric biopsy findings were normal, and there was normal acid secretion on histamine stimulation.

In Cases VI and VII, the two patients with grossly abnormal jejunal mucosa illustrate the value of jejunal biopsy in determining whether the malabsorption is due to a primary small-bowel lesion. In contrast, we investigated a case of steatorrhœa caused by fibrocystic disease of the pancreas, reported elsewhere by Marks and Anderson (1960). In this the jejunal biopsy was normal.

CASE VIII.—The diagnosis in this case was Whipple's disease ("intestinal lipodystrophy"). A farmer, aged 44 years, suffering from chronic diarrhœa, loss of weight, anæmia and pigmentation, was referred by Professor E. G. Saint, of the University of Western

FIGURE XII

Case VII. Jejunal biopsy in intestinal malabsorption, from a female, aged 70 years, with steatorrhœa, anæmia and osteoporosis. Surface view shows absence of villi as in Case VI. (Unstained, ×25)

Australia, with the established diagnosis of Whipple's disease. This had been determined at laparotomy by the microscopic appearance of the bowel, distended lymphatics and enlarged mesenteric glands. Examination of sections of a mesenteric gland revealed the classical picture of cells staining with periodic acid-Schiff stain (P.A.S.) and sinuses distended with fat (Taft *et alii*, 1959). The patient improved somewhat with steroid therapy.

He then visited his sister in Victoria, and while there suffered a severe relapse with diarrhœa, anæmia, œdema and hypoproteinæmia. He was admitted to our Unit, where a small-bowel biopsy showed a gross abnormality. On the surface view there was great thickening of the villi (Figure XIII). Examination of sections confirmed the swollen villi, distended with the characteristic glycoprotein-containing cells, identified by their deep staining by the P.A.S. method (Figure XIV). A second biopdy, performed two weeks later to assess the value of treatment, gave similar findings.

The small-bowel biopsies in Case VIII emphasize the opinion of Shiner (1959) and others that this procedure is the most valuable method for diagnosing Whipple's disease. It avoids a laparotomy, as the pathognomonic features are invariably present in the small bowel.

FIGURE XIII

Case VIII. Jejunal biopsy in Whipple's disease, from a male, aged 44 years, with diarrhœa, cachexia, anæmia and pigmentation. Surface view, showing swollen villi. (Unstained, ×40)

DISCUSSION

The present study with the suction small-bowel biopsy tube was based on 51 successful biopsies performed on 46 patients suffering from a wide variety of diseases. The results of the study support the work of Shiner (1959), Culver *et alii* (1959) and others that small-bowel biopsy is neither distressing nor hazardous to the patient and is a most valuable investigation in some forms of gastro-intestinal disease, particularly when the cause of intestinal malabsorption with steatorrhœa, anæmia, loss of weight, vitamin deficiency or osteoporosis is being sought.

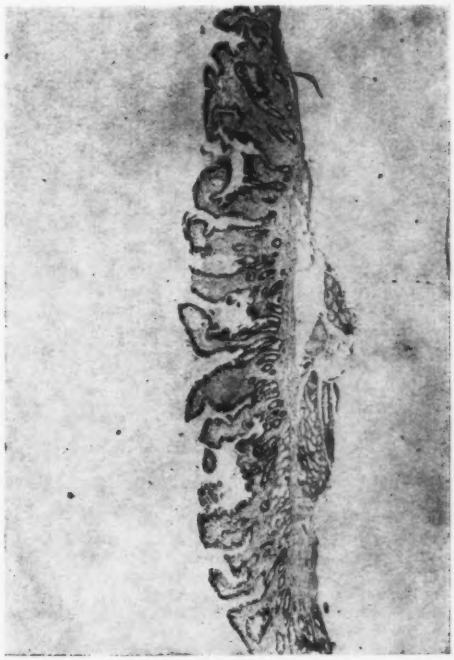

FIGURE XIV

Case VIII. Jejunal biopsy in Whipple's disease (see Figure XIII). Section shows the classical swollen villi distended with cells. These cells reacted positively with P.A.S. stain, typical of Whipple's disease. (Hæmatoxylin and eosin stain, ×24)

Thus it may aid in determining whether steatorrhœa is due to changes in the absorptive surface of the small bowel, or to other causes such as pancreatic deficiency or shunts. It is also of considerable value in the diagnosis of Whipple's disease, the characteristic widespread infiltration of the mucosa cell staining with P.A.S. being confined to this disease.

Our limited studies also indicate that when serial gastric biopsies reveal that the patient is suffering from chronic gastritis which over the years may progress to gastric atrophy, then the upper part of the small bowel does not reflect a

similar degenerative change. There is neither pronounced cellular infiltration nor atrophy. On the other hand, in our two cases of the malabsorption syndrome with gross atrophy of the intestinal villi, the corresponding gastric biopsy findings and gastric secretion after histamine were normal. This is in keeping with the more extensive experience of Shiner.

The cause of both chronic atrophic gastritis and the malabsorption syndrome is unknown. This lack of correlation between the histological changes in the stomach and small bowel in these two conditions suggests they have not a common causal factor.

ACKNOWLEDGEMENTS

We wish to thank the members of the Honorary Medical Staff of the Royal Melbourne Hospital for kindly referring patients, and the members of the Clinical Research Unit for their help, particularly Dr. S. Weiden, Dr. I. Maddocks, Sister I. Langford and Miss E. Earle. Mr. E. Matthaei, of the University of Melbourne, assisted with the photography.

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GASTRIC ULCER : AGE, SEX, AND A CURIOUS RETROGRESSION[1]

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From Sydney Hospital, Sydney

SUMMARY

The incidence of chronic gastric ulcer increases with age in both sexes.

A curious retrogression has occurred in Australia since 1945, involving a sudden exacerbation of chronic gastric ulcer in young women. At the present time women up to the age of 40 years are more likely to die from gastric ulcer than men of the same age. No similar change is yet apparent in other countries. It is probable that an alteration in the environment has been responsible.

THERE is general agreement that shortly after the commencement of this century gastric ulcer ceased to be a relatively common disease in young women in Europe and the United States of America. Previously, all available information indicated that women were more prone to gastric ulcer than men, but subsequently both mortality and incidence figures have shown an unvarying predominance of men at all ages.

This report deals with a retrogression which appears to have become manifest in Australia since 1945, as shown by an increasing incidence of chronic gastric ulcer in young women, so that both the incidence and the mortality of this disease up to the age of 40 years are greater now in women than in men.

MATERIAL

A survey was initially made of the records of gastric ulcer in patients admitted to the public beds of four general teaching hospitals in Sydney during the period 1945–1955. Cases were accepted when there was evidence of an open chronic gastric ulcer proved histologically from necropsy or resection material. A radiological diagnosis was accepted only when there was a definite report of a gastric ulcer crater without other features suggesting a neoplastic basis. Cases in recent migrants to this country, other than from the British Isles, were excluded. When an individual had more than one hospital admission, the recorded age was that at the first admission, except when resection or necropsy was performed, when the age at the time of these events was used. Cases of perforation were excluded, except when necropsy confirmed the presence of a chronic gastric lesion. Subsequently, with the use of the same criteria, a survey of records from two of these hospitals was made for both sexes for the period 1930–1939, and separately for women only during the period 1940–1945. An estimate of relative prevalence according to age was derived in each sex from the numbers obtained in each group divided by the number in the Australian population of that age in 1951, 1935 and 1943 respectively. The units in which relative prevalence is expressed are not comparable for the three periods.

RESULTS

The results are shown in Table I, and as frequency polygons in Figures I and II.

A difference in the age distribution of female subjects between 1930–1939 and 1945–1955 is

[1] Received on October 12, 1959.
[2] Honorary Assistant Physician.

TABLE I

Chronic Gastric Ulcer: Sydney Age Distribution of In-Patients

Period	Sex	Age Groups (Years)								Sex Ratio
		15 to 24	25 to 34	35 to 44	45 to 54	55 to 64	65 to 74	75 and Over	Total	
1945–1955	Male ..	0	30	129	207	256	131	20	773	1·3 / 1
	Female ..	15	103	171	126	116	69	15	615	
1930–1939	Male ..	1	18	69	99	88	28	1	304	2·5 / 1
	Female ..	3	8	25	36	35	15	1	123	
1940–1945	Female ..	2	7	11	20	27	12	3	82	

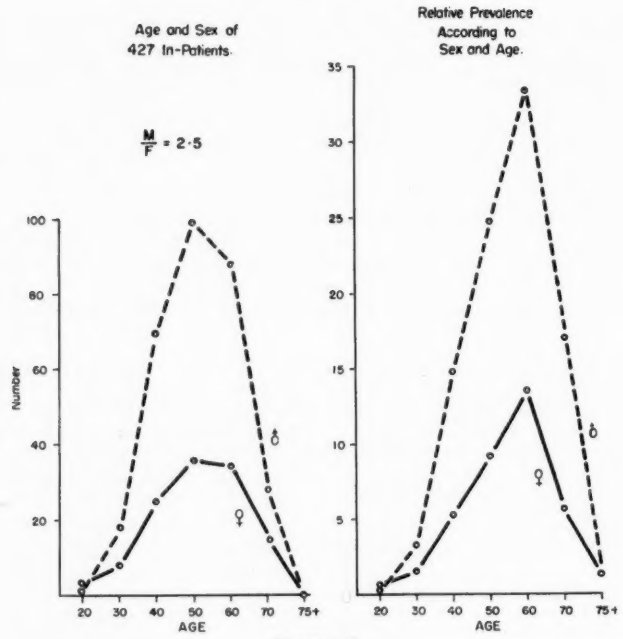

FIGURE I

Age and sex of 427 in-patients with chronic gastric ulcer in Sydney, 1930–1939, with relative prevalence according to age in each sex

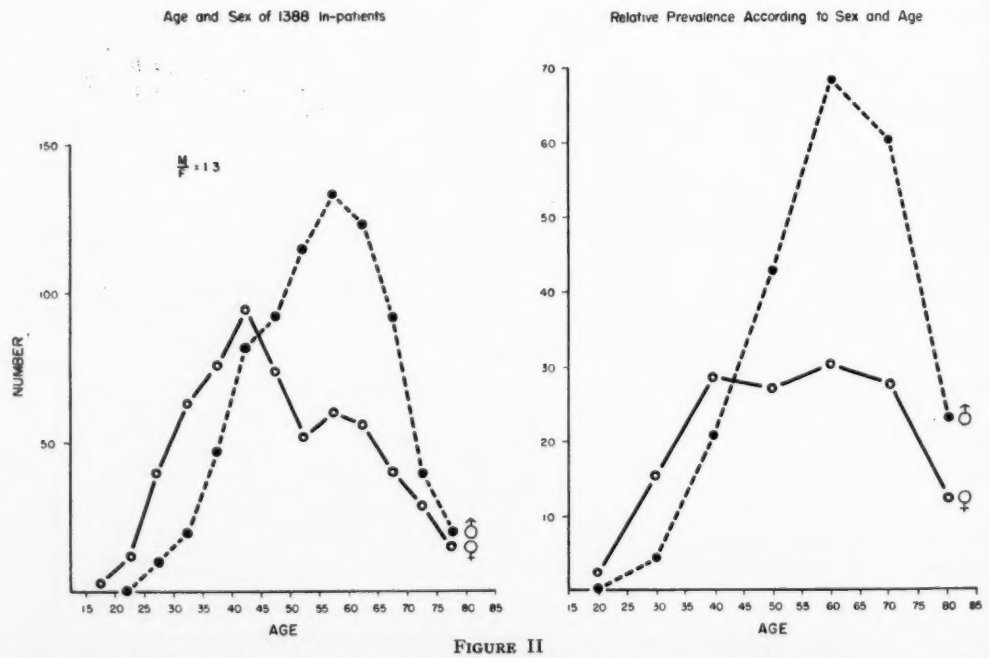

FIGURE II

Age and sex of 1388 in-patients with chronic gastric ulcer in Sydney, 1945–1955, with relative prevalence according to age in each sex

evident, although there is no great difference in male subjects. The increase in females in the younger age groups has converted the male to female ratio from 2·5 in 1930–1939 to 1·3 in 1945–1955. During the period 1940–1945

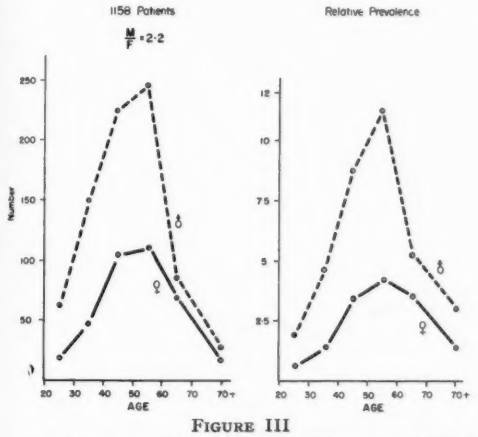

FIGURE III

Age and sex of 1158 in-patients with gastric ulcer in England, 1938 (Bell, 1940), with relative prevalence according to age in each sex

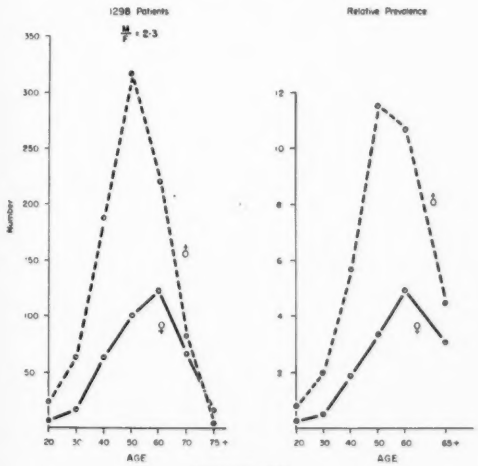

FIGURE IV

Age and sex of 1298 in-patients with gastric ulcer in England, 1949 (McKay, 1951), with relative prevalence according to age in each sex

only 82 female subjects were included, but it is clear that the change in age distribution was not present then.

For comparison, figures of the age distribution of gastric ulcer in-patients in England are shown graphically in Figures III and IV. Both series (Bell, 1940; McKay, 1951) represent large numbers of cases admitted to several English hospitals during 1938 and 1949. Cases of perforation are known to have been excluded from the 1949 series. It is evident that no great change has occurred in England in the age distribution in either sex, nor has the sex ratio altered between the two periods of observation, which correspond approximately to the pre-war and post-war Sydney series. The relative prevalence figures for males in all four series are very similar, with a steady rise to the age of 60 years. For females the two English series correspond to the 1930–1939 Sydney series, again showing a rising prevalence with age to a peak at 60 years. Figure V shows the relative prevalence of gastric ulcer in Sydney during the three periods of observation and demonstrates the remarkable rise in incidence in young women since 1945.

TABLE II

Age Distribution of Gastric Ulcer in Out-Patients Diagnosed by Barium-Meal X-Ray Examinations, Sydney, 1953

Age Group (Years)	Male Subjects		Female Subjects	
	Gastric Ulcer Found	Number Examined	Gastric Ulcer Found	Number Examined
15 to 24	0	45	2	36
25 to 34	4	82	7	101
35 to 44	5	120	13	137
45 to 54	10	111	3	125
55 to 64	7	110	3	105
65 to 74	7	81	1	68
75 and over	2	29	4	18
Total ..	35	578	33	590

In dealing with chronic ulcer it could be argued that the age distribution of hospital cases may not represent the true age incidence of the disease. It is extremely unlikely that routine hospital in-patient records could give an accurate estimate of attack rate at different ages; but the present method of recording age on admission to hospital at least provides a uniform method for comparison of material from several sources at different times. It is possible that the proportion of complications in one series may differ from that in another, and possibly also the age distribution. In Sydney, a review of 1200 barium meal X-ray examinations on out-patients in 1953 at one hospital (Table II) shows that for uncomplicated gastric ulcer the sex difference in age distribution is similar to that described for the 1945–1955 in-patients, which include a large proportion of complicated cases. Table III records the ages (mean with standard

error) of in-patients with chronic gastric ulcer in Sydney in the period 1945–1955, classified by sex, by method of selection and also by the blood group, to compare with the data provided by Aird *et alii* (1954) for England during the period 1948–1953. For each sex in Sydney, there is no difference in the mean age incidence of chronic gastric ulcer, between those cases diagnosed by radiology and those diagnosed at operation or necropsy, but there is a distinct sex difference in the mean ages by both methods of selection. During the period 1945–1955 in Sydney, the sex difference in age distribution of chronic gastric ulcer therefore appears to be a valid observation. Since 1945, the mean age incidence of surgical gastric ulcer in males is similar in the two countries; but whereas the mean age of female patients is the same as that of male patients in England, it is distinctly lower in Sydney.

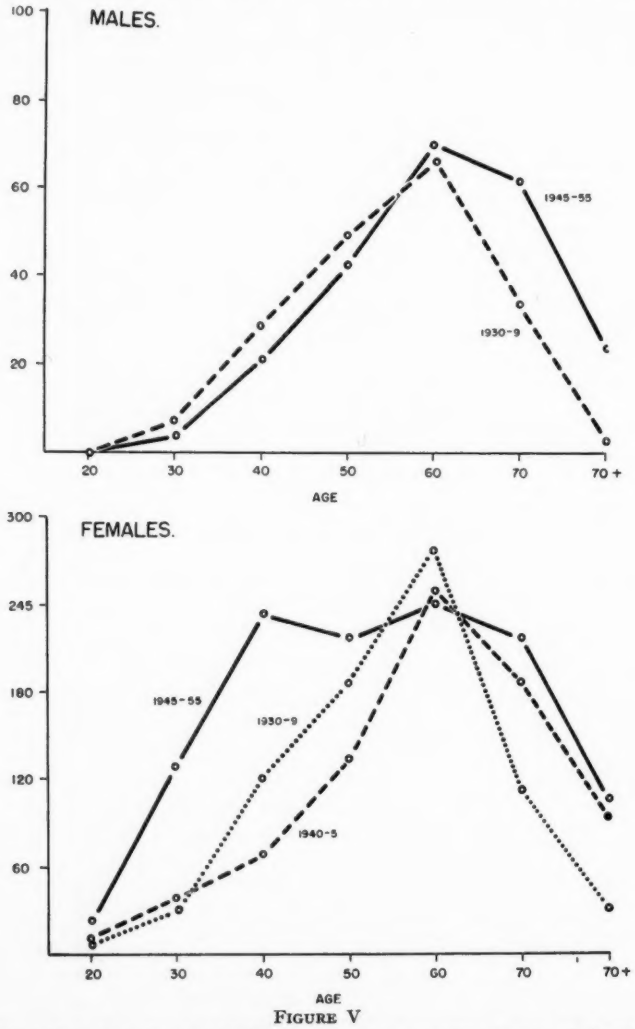

FIGURE V

Comparison in each sex of relative prevalence according to age of chronic gastric ulcer in Sydney in-patients, 1930–1939 and 1945–1955, including females, 1940–1945. The units are arbitrary. Above, males; below, females

TABLE III

Chronic Gastric Ulcer. Age by Sex and Blood Group
(Means with standard error)

Place	Source	Method of Selection	Male Subjects					Female Subjects				
			Number	A		O		Number	A		O	
				Mean (Years)	S.E.[1]	Mean (Years)	S.E.		Mean (Years)	S.E.	Mean (Years)	S.E.
England (1948–1953)	Aird *et alii* (1954)	Macroscopic	591	54·19	0·69	52·19	0·63	323	56·48	1·16	55·20	0·90
Sydney (1945–1955)	Present series	Necropsy and Resection	313	55·10	0·85	54·02	0·85	282	48·14	1·18	45·21	1·09
		X-ray	160	56·30	2·00	55·83	1·13	93	51·26	1·97	49·27	2 04

[1] Standard error.

Mortality Evidence

Comparisons based on hospital figures alone may be misleading, and it is pertinent to observe that mortality data for Australia support the observations made from hospital cases in Sydney.

A comparison of the age-specific mortality rates from gastric ulcer per 100,000 living in Australia and in England and Wales during the period 1951–1953 is shown in Figure VI. The figures for males from both countries show a parallel trend, but for females, the mortality line shows a distinct hump in Australia, which was not present in mortality data up to 1944. In assessing the significance of this rise in female gastric ulcer mortality in the younger age groups, the mean mortality rates for Australia from gastric ulcer during the periods 1940–1945 and 1950–1955 have been compared in cohort fashion (Figure VII). At all ages under 60 years, a pronounced fall in male gastric ulcer mortality has occurred in Australia. A similar change has occurred in England in both sexes. Females in Australia have not shared in this fall, and there has been a pronounced rise with cohorts of 1913 and later. Figure VIII shows that the inflection caused by the rising mortality rate in younger women has advanced 20 years during the 10 years of observation.

DISCUSSION

From the data of England in 1922–1937 (Tidy, 1945), 1938 and 1949, in which large numbers of hospital cases are recorded, it is apparent that the incidence of gastric ulcer increases with age at a relatively constant rate in each sex, and this has also been shown in a recent study on morbidity in general practice (Medical Research Council, 1958). The Sydney figures for 1930–1939 show the same pattern. Although the actual figures show a decline in the relative prevalence after the age of 60 years in hospital cases, this does not necessarily imply a fall in incidence. The decline occurs, for example, with gastric carcinoma, in which Case (1956) has shown that the generation age-specific mortality still continues to increase with ages

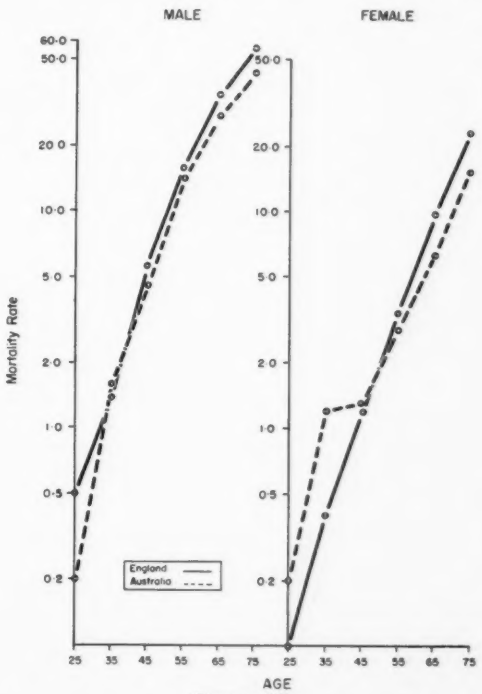

FIGURE VI

Mean age-specific mortality rates from gastric ulcer in 1951–1953 in England and Australia in each sex. The rates are expressed as deaths per 100,000 living at age

over 60 years. The excellent figures of all hospital discharges provided by the New Zealand Department of Health for 1954 and 1955 show that the relative prevalence of gastric ulcer continues to increase with age after 60 years in each sex in that country (Figure IX).

the age of 25 years had been reached. This difference between gastric and duodenal ulcer in relation to the age has also been demonstrated in necropsy material in Great Britain in 1956. By the use of cases of active chronic ulcer discovered as an incidental finding, the incidence

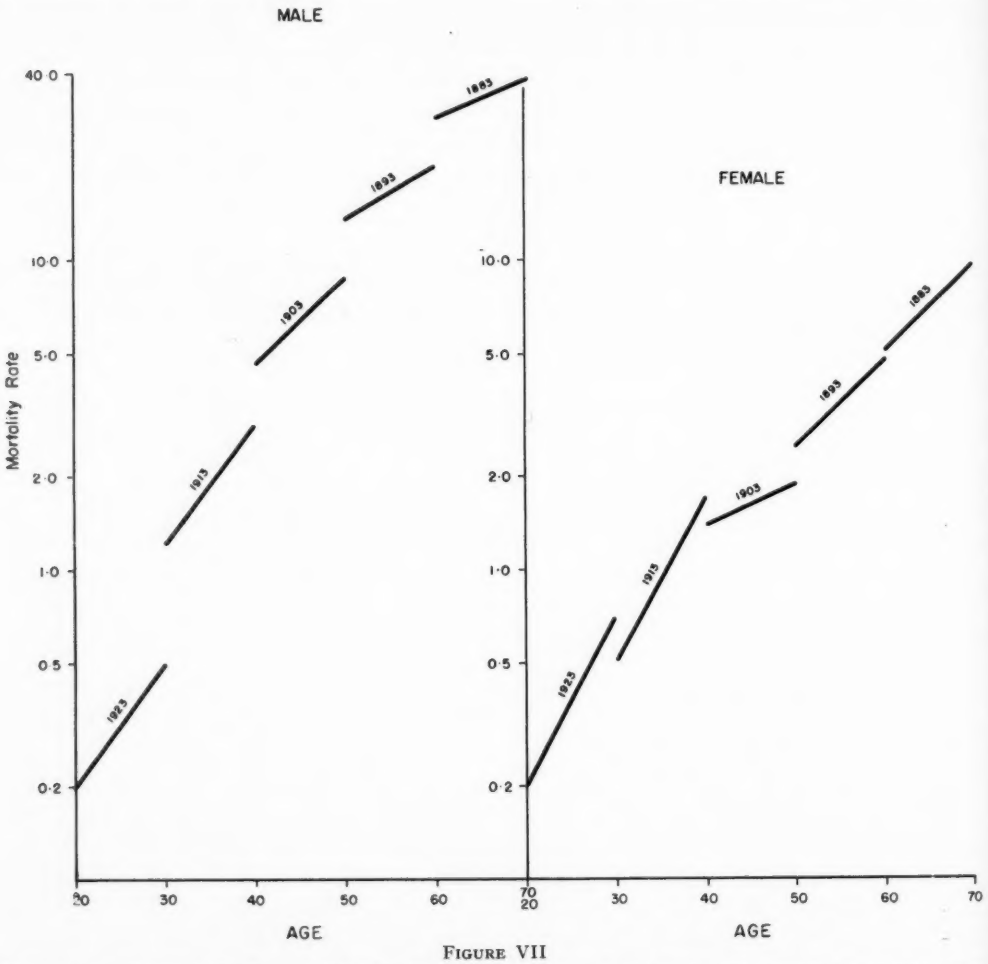

FIGURE VII

Mean age-specific mortality from gastric ulcer in Australia, 1941–1945 and 1951–1955, arranged in cohorts. The rates are expressed as deaths per 100,000 living at age

If the incidence of chronic gastric ulcer increases with age, this may depend on simple cumulative frequency, or on a rising attack rate, or on both. It is of interest that Baron and Vaughn Jones (1958), dealing with men in the British Army in 1955, showed an attack rising with age for gastric ulcer, but a relatively constant attack rate for duodenal ulcer after

of gastric ulcer increases with age in both sexes after 35 years, while that of duodenal ulcer does not (Watkinson, 1958); this corresponds with the experience of Matthew Stewart in Leeds in 1930–1949 (Watkinson, 1957).

When one is dealing with chronic disease, rising age-specific mortality figures may not necessarily parallel the age incidence, but

merely reflect the increasing tendency of any population to die with the passage of years. It is of unusual interest that since 1945, in Australia, young but not older women have shown a rise in both relative prevalence and mortality figures relating to gastric ulcer. From the data available, it is not possible to decide whether the falling mortality from gastric ulcer in Australian and English males from 1945 onwards has been associated with a similar fall in incidence of the disease. The relative prevalence patterns according to age in Sydney men (Figure V) indicate that no change has occurred between 1930–1939 and 1945–1955, and if a fall in incidence has occurred, it has affected men of all ages under 60 years.

In the period 1950–1953, an analysis of the standardized mortality rates for gastric ulcer prepared by Segi *et alii* (1957) shows a good correlation between the male and female rates from 19 countries, the male rates being on the average 66% higher than the female rates. No similar information has been prepared relating to earlier periods; but it is apparent that up to the first decade of this century the female incidence of gastric ulcer was higher than the male. Young women particularly were affected, with a pronounced tendency to hæmorrhage and perforation. It has been assumed by many subsequent writers that the lesions present in young women then were acute rather than chronic peptic ulcers. This is difficult to ascertain. Brinton (1865) stated that "this epoch predisposes not so much to the occurrence of this lesion as to a peculiar character and termination of it", that there was "a

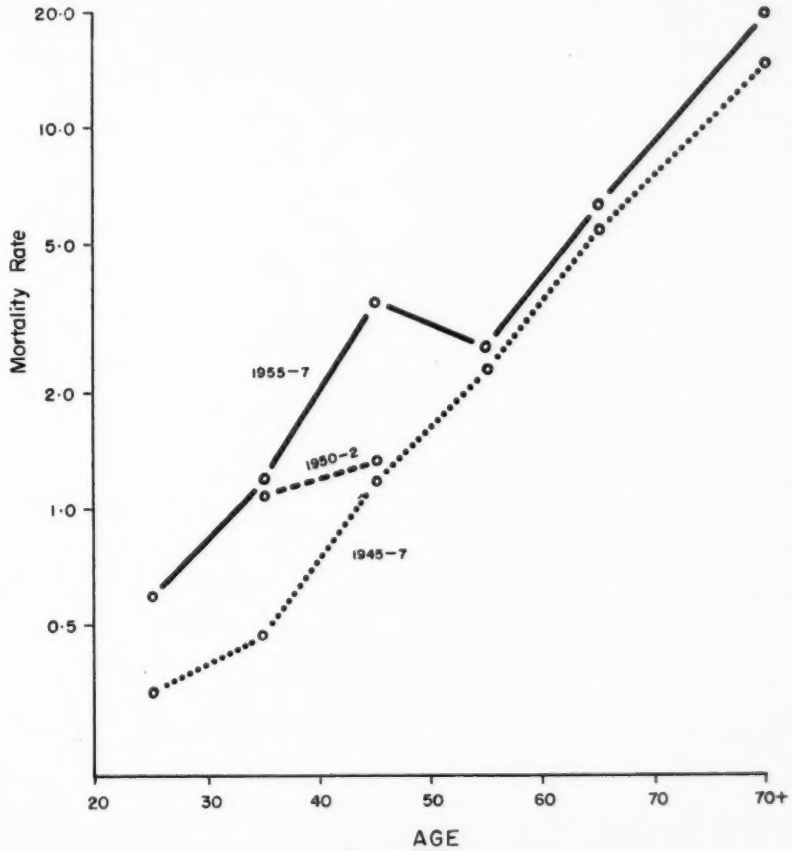

FIGURE VIII

Mean female age-specific mortality rates from gastric ulcer in Australia, 1945–1947 and 1955–1957. The rates between the ages of 30 and 50 years for 1950–1952 are interpolated. The rates are expressed as deaths per 100,000 living at age

tendency to perforation rather than a proneness to ulceration", and finally that "there will still be such a preponderance in the total formed by the gastric ulcers of the male and of the middle-aged and aged females as to exclude the above group from any general significance in the ætiology of the disease". Hurst (1929) pointed out that it was not until 1897 that Dieulafoy made for the first time a clear-cut pathological distinction between acute and chronic gastric ulcers, so that information before 1900 is likely to be misleading. Although

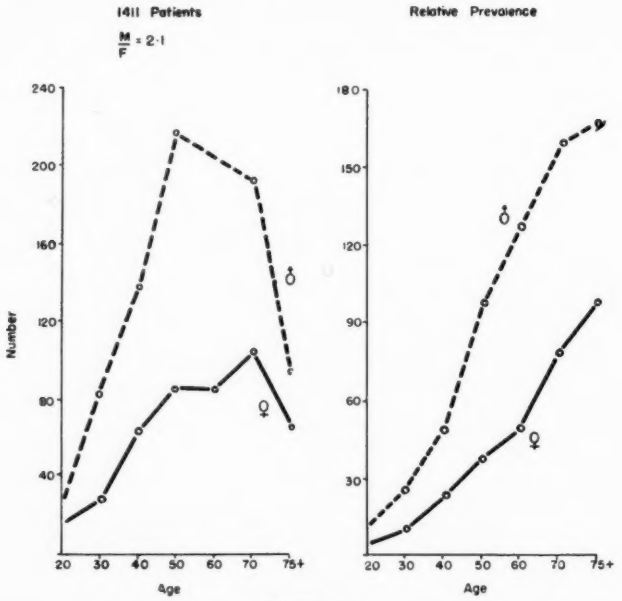

FIGURE IX

Age and sex of 1411 in-patients with gastric ulcer in New Zealand, 1954–1955 (New Zealand Department of Health, 1957), with relative prevalence according to age in each sex

Hale-White (1906) concluded that the common cause of hæmatemesis in young women was an acute rather than a chronic ulcer, Bolton (1913) gave the figures of F. C. Grieg concerning the previous decade at University College Hospital, London, where chronic gastric ulcer showed a sex difference in the age distribution of cases, with an excess of females under the age of 40 years. This appears to be the last documented report of female supremacy in gastric ulcer at any age. In Sydney, the evidence shows that the chronic lesion has accounted for the reappearance of gastric ulcer in young women.

Many writers have ascribed the disappearance of gastric ulcer in young women early in the century to the decline of stays and tight-lacing among the appurtenances of fashion, and this view appears to have the authority of common consent (Ivy *et alii*, 1950). No similar modish devices appear to have been introduced into Australia in recent years, and perhaps herein lies the curiousness of the retrogression.

No obvious differences in the clinical behaviour of gastric ulcers in young women have been observed to distinguish them from other cases. A comparison of the ABO blood group distribution in female subjects below the age of 35 years and over 60 years shows no difference. It has been suggested previously (Billington, 1959) that there is a sex difference in the intra-gastric site affected by chronic peptic ulceration. In the 1945–1955 series, the estimated site of ulceration shows no difference between old and young females; both age groups differ from males in having a higher proportion of lesions located above the radiological angulus.

Three general possibilities to explain the phenomenon in Australia are suggested by the data: firstly, a population change; secondly, immigration; thirdly, "ulcerogenic" change.

From cohort mortality figures it could be postulated that, commencing suddenly about 1908, and subsequently, a population of women

was born who later were to become at least twice as prone to die of gastric ulcer as those born prior to this date. In this female population, the rate of developing and dying of gastric ulcer might increase with age along lines similar to those of women born prior to 1908, and of men. The full effect of this phenomenon would be expected in the decade 1970–1980, when those women would have reached the age by which most of their chronic gastric ulcers would have occurred. At this time there would be little sex difference in the age distribution of chronic gastric ulcer cases; but there would be a complete reversal of the sex ratio, with more women affected than men, and as many women dying of gastric ulcer as men. Three objections may be raised to this hypothesis. The first relates to the continuous large-scale migration which has been taking place to this continent almost continuously throughout the half century, and therefore cohort studies may not be valid. It is a fact, however, that until 1946 there was no change in the immigration policy, and migrants were derived largely from the British Isles, as had been the case prior to 1908. Only subsequent to 1946 has a larger proportion of migrants come from the continent of Europe, mainly its eastern and Mediterranean regions. As no similar change is demonstrated in the cohort analysis of male gastric ulcer mortality, it seems likely that the cohort method is valid. The second objection relates to the female mortality figures for gastric ulcer, in which the sudden change in mortality rate according to age appears to be advancing more quickly than can be accounted for by the rising age of a new population at risk (Figure VIII). The third objection relates to the observation that in both the 1930–1939 and the 1940–1945 periods, the age distribution of chronic gastric ulcer in female in-patients did not show an increase of cases in those age groups which would correspond to 35 to 55 years in 1945–1955, either in the absolute or in the relative figures. Any attempt to make the date of the possible population change later than 1908 meets further objections on similar grounds and cannot explain the cohort mortality data.

The effect of immigration on the Australian population is difficult to determine. Parker and Walsh (1958) have shown that the immigration has not yet altered the blood-group distribution in blood donors in Sydney in either sex. It is unlikely that immigration is responsible for the change in the age distribution of female in-patients with chronic gastric ulcer, since the 1945–1955 series contained none who were not born in Australia or the British Isles, where no similar change appears to have occurred. Mortality data support the argument, as the female mortality rate from gastric ulcer up to the age of 45 years in the British Isles is less than the present Australian figures, and as the male mortality rates under the age of 60 years have fallen uniformly in both countries since 1946.

The most satisfactory general possibility to account for the present pattern of gastric ulcer is that an exogenous change has occurred, resulting in the occurrence of an increased number of chronic gastric ulcers in young women after World War II. It is of interest that Linn (1946), in Adelaide, South Australia, demonstrated during the period 1939–1944, on in-patient material, excluding perforations, that the age distribution of female chronic gastric ulcer subjects was similar to the 1930–1939 and 1940–1945 series in Sydney. Recently, Saint (1958), in an excellent survey of peptic ulcer in Western Australia in 1955–1956, using in-patient, out-patient and private patient material, showed the same sex difference in the age distribution as this paper describes for Sydney in the period 1945–1955. It seems, therefore, that the increased incidence of chronic gastric ulcer in young women is nation-wide and has occurred since 1945. The possible state of affairs in 1945–1955 could be represented graphically in Figure X, in which the relative prevalence in women is bi-modal, an " ulcerogenic " influence acting to cause a new female ulcer population in the child-bearing and premenopausal years. It cannot be decided whether this influence is new or merely the reintroduction of factors present in Europe prior to 1910.

It is possible that this will not prove to be a permanent state of affairs. The changing mortality figures for women since 1945 (Figure VIII) suggest that the rising rate is advancing with age and ahead of the initial population affected. If present trends continue, it may be anticipated that by 1965 the increased female mortality rate from gastric ulcer will include the 60–70 years age group, and for all ages the female mortality rate will be approximately double that existing in the period 1940–1945. It may equal and perhaps exceed the male mortality if the latter continues to fall. By 1965, under these circumstances, the age distribution of chronic gastric ulcer will cease to show a significant sex difference, and in both sexes the age pattern existing in the period 1930–1939 will be restored, with women exceeding men in total numbers.

Consideration of data from other countries is of interest. The rising mortality from gastric ulcer in young women observed in Australia appears not to have occurred in England and

Wales (to 1956), in the U.S.A. (to 1956), in Denmark (to 1957), in Norway (to 1955), in New Zealand (to 1955), or in the 12 other countries reported up to 1953 by the World Health Organization (1955). An up-to-date study of the age and sex distribution of gastric ulcer and its relative prevalence in other countries is restricted by the paucity of available or published information. No sex difference in relative prevalence according to age in clinical cases has been observed in data from England,

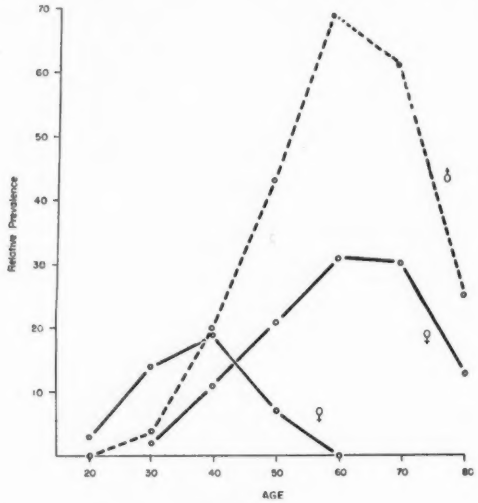

FIGURE X

Possible interpretation of the relative prevalence according to age of chronic gastric ulcer in Sydney in-patients, 1945–1955, derived from Figure II. The relative prevalence figures in females may be divided into two populations, one with an age distribution similar to males, the residuum constituting a new population in the child-bearing and pre-menopausal years as a result of a possible ulcerogenic influence described in the text

1949 (McKay, 1951), 1941–1954 (Avery Jones, 1956), 1948–1953 (Aird *et alii*, 1954); from Glasgow, Scotland, 1946–1948 (Jamieson *et alii*, 1949); from Molde, Norway, 1938–1954 (Ohma, 1957); from New Zealand, 1954–1955 (New Zealand Department of Health, 1957); from Stockholm, Sweden, 1938–1952 (Tomenius, 1955); from Denmark, 1948 (Alsted, 1953); from Switzerland, 1956 (Werthemann and Huber, 1957); from Rhode Island, U.S.A., 1946–1955 (Bernardo *et alii*, 1958); from Japan, 1953–1955 (Kurokawa and Ishikawa, 1958); from Austria, 1958 (Boller, 1959); from Brazil, 1957 (Siffert, 1959); from Johannesburg, 1957 (Schade, 1958). A report from Italy (Balestra, 1958) suggests that there may be a sex difference in the age distribution of cases, but mortality data to 1954 do not lend support to this suggestion. Necropsy data are even more restricted and difficult to interpret. The figures from Great Britain during the national survey in 1956 reported by Watkinson (1958) show no sex difference in the age distribution of chronic gastric ulcer when related to death or as an incidental necropsy finding, nor has any recent change occurred when these figures are compared with those given by Matthew Stewart at Leeds (Watkinson, 1957).

It seems clear, therefore, that the change in the age distribution of chronic ulcer in women in Australia has so far not been evident either in men in Australia or in men or women in other countries. It is probable that an environmental alteration has been responsible, initially affecting a generation of women in their child-bearing and premenopausal life and becoming manifest quite suddenly after 1945. It is not known whether the change has involved the addition of a new factor or the exacerbation of one already existing, or whether a previous possible protection has declined. From the information available, no inference can be drawn as to whether men have not been affected by the alteration or are constitutionally unable to react to it. Finally, as this survey deals with chronic ulcer, there is no evidence to decide whether the change relates to the genesis of gastric ulceration or to factors affecting healing or the promotion of chronicity.

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THE EFFECTS OF DIET ON THE BLOOD COAGULATION RESPONSE TO ALIMENTARY LIPÆMIA IN HEALTHY AND ATHEROSCLEROTIC MEN[1]

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SUMMARY

The response of blood clotting to a standard meal of fat was measured in nine healthy young men and in 10 atherosclerotic older men when the serum lipids in the fasting state were maintained at their usual levels, and after they had been lowered by dietary manipulations.

Ingestion of the test meal was followed by an acceleration of clotting in the "Stypven" test and the whole-blood clotting time (W.B.C.T.), a rise in plasma turbidity, and increases in the concentrations of serum total esterified fatty acids and serum phospholipids. There was no change in the serum cholesterol level.

Lowering of the fasting serum lipid levels had no effect on the "Stypven" response in any of the subjects, and had no effect on the response of the W.B.C.T. to the test meal in the healthy controls. However, both the fasting and post-prandial W.B.C.T. in the atherosclerotic subjects were longer when the serum lipid levels were low.

The presence or absence of atherosclerosis was without effect on the response of the "Stypven" test or the W.B.C.T. to alimentary lipæmia, except that lowering of the fasting lipid levels produced a longer post-prandial W.B.C.T. in the atherosclerotic subjects.

There were statistically significant associations between blood clotting and the serum lipid levels and plasma turbidity. In healthy subjects, acceleration of clotting in the "Stypven" test was associated with a rise in plasma turbidity and serum total fatty acid levels, while the W.B.C.T. became shorter as the serum phospholipid levels rose. There was no significant relationship between the two clotting tests. On the other hand, in the atherosclerotics the W.B.C.T. varied with the "Stypven" time, and clotting in both tests was accelerated as the concentrations of particulate and non-particulate fat increased.

It was observed that the longer the W.B.C.T. in the fasting state and the lower the fasting serum lipid levels, the greater was the response of clotting in this test to the fat meal.

THE effect of alimentary lipæmia on blood coagulation has been the subject of many investigations, but opinion is still divided as to precisely what happens. Some measures of clotting, such as the "Stypven" response, regularly undergo an acceleration when fat is ingested, while others, such as the W.B.C.T., have given contradictory results in the hands of different workers.

Attempts have also been made to demonstrate an association between the clotting response to fat-containing meals and the presence of complications of atherosclerosis, but here also there are differences of opinion. Mustard (1958) found that a greater acceleration of clotting occurred in atherosclerotic people compared with healthy controls, while O'Brien (1958) could find no difference. If Mustard's findings are correct, they indicate an enhanced tendency to fibrin deposition and arterial occlusion in this disease. If these differences in blood clotting responses are real, what factors are responsible? Possible influential factors which must be considered include the rate at which fat is cleared from the blood, the concentration of blood fats in the fasting state and, perhaps, the fasting clotting time, which may influence the degree to which clotting can be accelerated.

An investigation into the effects of diet on the serum lipids and blood clotting in the fasting state (Goldrick, 1960), provided an opportunity to study the factors influencing the response of blood coagulation to alimentary lipæmia. Measurements of lipids and blood clotting were carried out at weekly intervals in healthy young men and in atherosclerotic older men. Observations were made during a control period of three weeks when the subjects ate their usual diets, and during a period of four weeks (test period) when the serum lipid levels were reduced by substituting unsaturated fat (sunflower-seed oil) for an equivalent amount of saturated fat in

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[2] Research Fellow, supported by an Anonymous Family Trust.

the diet. Twice during the control period and once during the test period, a meal of cream was given to each subject, and serum lipid and blood clotting measurements were made before and at intervals after the meal.

MATERIALS AND METHODS

A full description of the subjects and the dietary methods used to reduce the serum lipid concentrations has been given in another communication (Goldrick, 1960).

Serum Lipid and Blood-Clotting Determination

Measurements were made of the W.B.C.T. in siliconed tubes, the "Stypven" clotting time, the plasma turbidity, and the serum total cholesterol, phospholipid and esterified fatty acid contents. With the exception of the plasma turbidity determination, all estimations were carried out on duplicate samples. The methods for measuring blood clotting and serum total cholesterol and phospholipid contents have been described elsewhere (Goldrick and Whyte, 1959).

The total esterified fatty acid contents were determined by the method of Stern and Shapiro (1953). The standard deviation of the differences between duplicates was 0·69 mEq./l of serum, derived from the expression $\sigma_e = \sqrt{\frac{\Sigma d^2}{2n}}$.

For plasma turbidity, 0·5 ml. of platelet-poor plasma (as used in the "Stypven" test) was diluted to 3·5 ml. with normal saline, and the optical density was measured in a Beckman model DU spectrophotometer, against a normal saline blank, at a wave-length of 420 mμ.

The Fat Tolerance Test

Blood was collected by clean venepuncture with siliconed apparatus for serum lipid, plasma turbidity and blood-clotting estimations. The first venepuncture was performed with the subjects in the basal state. A meal of fat was then ingested, and further blood samples were taken one and a half, two and a half, four and a half and seven hours after the meal. Throughout the test the subjects remained at rest, in quiet surroundings, and no other food or drink was allowed.

The test meal comprised heavy cream, 180 grammes, milk, 120 grammes, apple, 90 grammes, and sugar, 15 grammes, and provided approximately 64 grammes of fat, 33 grammes of carbohydrate and 8 grammes of protein. The mixture was allowed to stand in the refrigerator for several hours prior to ingestion as it was more palatable when cold.

RESULTS

The changes customarily observed in the serum lipid levels, plasma turbidity and blood coagulation in response to the test meal of cream are shown in Figure I. For the purpose of illustration, the curves shown are those derived from the averages of nine fat-tolerance tests in atherosclerotic subjects when the diet contained sunflower-seed oil. It will be noted that as turbidity increased there was a rise in the concentrations of fatty acids and phospholipids, and an acceleration of clotting as shown by shortening of both the W.B.C.T. and the "Stypven" time. The serum cholesterol content remained essentially unchanged. Toward the end of the test, the clotting times were beginning to return to their original values, as, too, were the plasma turbidity and the concentrations of fatty acids and phospholipids.

While Figure I gives a representative picture of the changes which occurred in the fat tolerance test, certain differences were noted between the two groups of subjects and even in the same subjects during the different dietary regimens. In order to describe these differences, each item of the fat tolerance test will be considered separately.

The Whole Blood Clotting Time

Inspection of Table I reveals that ingestion of the test meal was followed by a definite and statistically significant acceleration of the W.B.C.T. in both groups of subjects during

TABLE I

Average Values for the W.B.C.T. (Minutes) in Healthy and Atherosclerotic Men, Together with the Standard Deviations and the P Values Wherever there are significant Differences between Fasting and Lipæmic Samples

Subjects	Dietary Period	Number of Observations	Fasting	At 1·5 Hours	At 2·5 Hours	At 4·5 Hours	At 7·0 Hours	Pooled Post-prandial Data
Healthy	Saturated fat	15	27·63±3·79	24·63±2·28 $P<0·02$	24·90±3·35	24·60±3·28 $P<0·05$	24·97±2·83 $P<0·05$	24·78±2·93 $P<0·01$
	Unsaturated fat	8	27·81±5·57	23·69±1·71	23·44±2·08	25·63±3·91	22·06±4·70	24·09±3·51 $P<0·05$
Atherosclerotic	Saturated fat	17	28·47±3·98	26·85±3·47	25·53±3·61 $P<0·05$	24·21±2·42 $P<0·001$	25·21±2·41 $P<0·01$	25·45±3·17 $P<0·01$
	Unsaturated fat	9	33·83±5·84	28·22±5·70	26·94±3·71 $P<0·02$	26·94±3·09 $P<0·01$	29·56±3·63	27·92±4·26 $P<0·01$

each of the dietary periods. Not all the individual averages were significantly shorter than the fasting values; but in every case when the post-prandial observations were pooled, a significant acceleration of clotting was demonstrated.

It will also be observed (Figure II) that there were no marked differences in the mode or extent of the W.B.C.T. responses between healthy and atherosclerotic subjects on ordinary diets, and that diet had no marked influence on the W.B.C.T. response in healthy subjects. Statistical comparisons of the fasting and pooled post-prandial averages representing these curves also revealed no differences.

However, it is clear that exhibition of unsaturated fat had a marked influence on the W.B.C.T. response to the test meal in atherosclerotic subjects. Both fasting and post-prandial clotting times were definitely longer during the consumption of sunflower-seed oil than when the diet contained mainly saturated fat. Analysis showed that the fasting clotting times differed at the 2% level of significance and that the post-prandial clotting times differed at the 1% level.

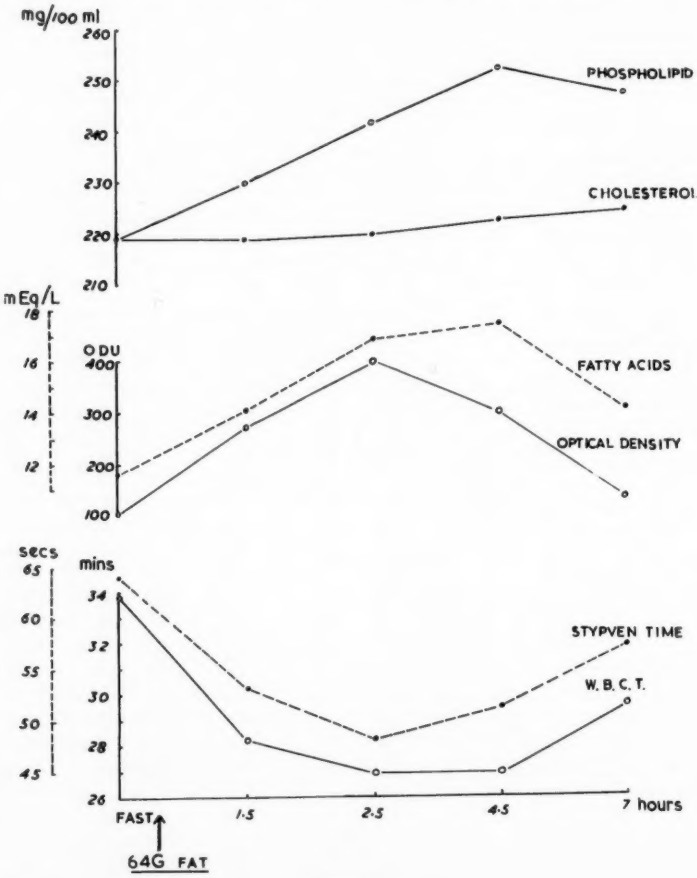

FIGURE I

An illustration of the changes in blood clotting, serum lipids and plasma turbidity after a test meal of cream

The post-prandial W.B.C.T. in atherosclerotic subjects was significantly longer in the sunflower-seed oil period than in the healthy subjects ($P<0{\cdot}001$). The fasting W.B.C.T. was also longer in the atherosclerotic group, but the difference was not significant, possibly owing to the small number of observations made in the fasting state.

The "Stypven" Time

A marked and statistically significant acceleration of clotting in the "Stypven" test followed ingestion of the test meal (Table II) in both groups of subjects and during each of the dietary periods. Not all of the individual averages were significantly different from the fasting clotting times, but in every case, when the post-prandial observations were pooled, a significant shortening of clotting could be demonstrated.

W.B.C.T. and the "Stypven" time. The calculations were made as follows: (i) percentage maximum changes:

$$\frac{\text{fasting clotting time—shortest post-prandial clotting time} \times 100}{\text{fasting clotting time}}$$

(ii) maximum absolute changes: fasting clotting time minus shortest post-prandial clotting time; (iii) differences at seven hours: fasting clotting time minus clotting time at seven hours. No significant differences ascribable to diet or disease were demonstrated.

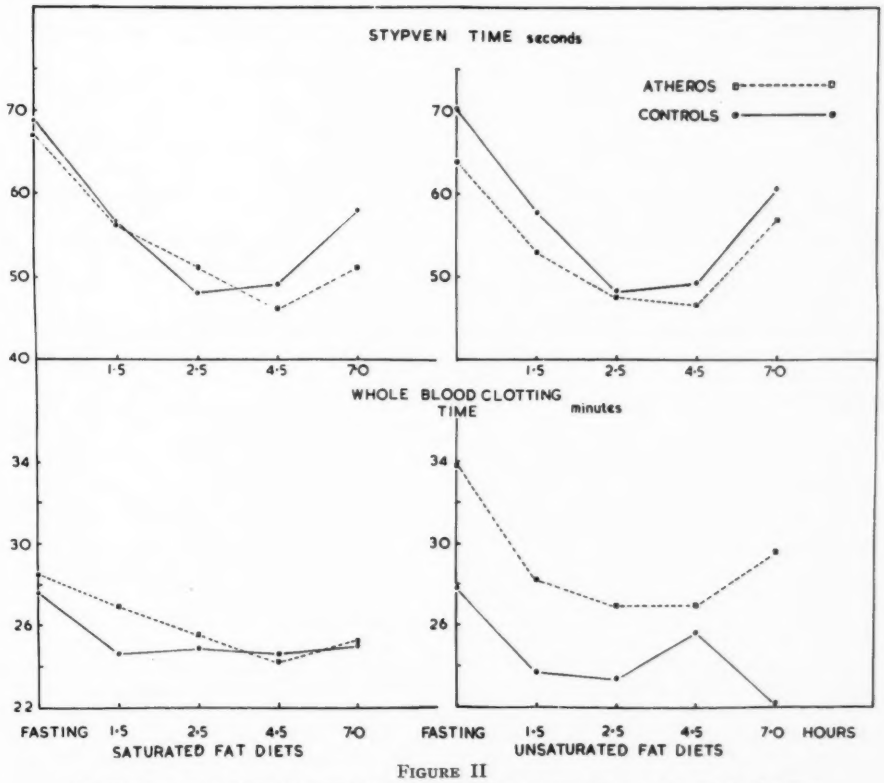

FIGURE II

The influence of dietary fats on the blood clotting responses to a test meal of cream

Analysis revealed that neither the presence or the absence of atherosclerosis nor the nature of the diet prior to ingestion of the test meal had any influence on the shape or magnitude of the "Stypven" response. This is also shown in Figure II.

Comparisons were also made between the groups of subjects and between the dietary periods in regard to the percentage maximum changes, the maximum absolute changes and the differences at seven hours for both the

Optical Density of Plasma (Figure III)

Ingestion of the test meal led to a marked increase in plasma turbidity, which was statistically significant in each group of subjects during both the dietary periods (Table III). The large standard deviations of the means indicate how variable was the response in different individuals.

TABLE II

Average Values for the "Stypven" Time (Seconds)

Subjects	Dietary Period	Number of Observations	Fasting	At 1·5 Hours	At 2·5 Hours	At 4·5 Hours	At 7·0 Hours	Pooled Post-prandial Data
Healthy	Saturated fat	15	68·80±16·44	56·87±16·77	48·13±17·42 $P<0·01$	49·20±16·65 $P<0·01$	58·07±17·85	53·07±17·74 $P<0·01$
	Unsaturated fat	8	70·25±13·77	57·88±17·34	48·38±17·10 $P<0·02$	49·38±17·56 $P<0·05$	60·88±19·04	54·13±18·57 $P<0·05$
Atherosclerotic	Saturated fat	17	67·12±12·67	56·71±13·24 $P<0·05$	51·29±12·16 $P<0·01$	46·29±11·57 $P<0·001$	51·35±14·10 $P<0·01$	51·41±13·32 $P<0·001$
	Unsaturated fat	9	63·89±6·73	52·89±8·03 $P<0·01$	47·56±9·26 $P<0·001$	46·67±7·11 $P<0·001$	57·11±10·56	51·06±9·78 $P<0·001$

The fasting optical density recordings were significantly lower in the atherosclerotics than in the healthy subjects during both dietary regimens, but no significant differences in pooled post-prandial averages could be demonstrated. There was no evidence to suggest delay in plasma clearing in the atherosclerotic group.

In neither group of subjects was there any significant difference in the fasting and post-prandial averages between the dietary periods. Consequently little weight can be placed on the suggestion from Figure III that the plasma turbidity was more intense in the atherosclerotic subjects when the background diet contained unsaturated fats.

Serum Total Fatty Acids Content

In both groups of subjects, irrespective of diet (Table III), the concentration of fatty acids increased significantly during the course of the fat tolerance tests.

Comparisons between groups and between diets in regard to the fasting and pooled post-prandial fatty acid levels showed several differences.

Figure IV indicates that the change to unsaturated fat in the background diet set the fatty acid response to the test meal at a lower level in both groups of subjects, but did not alter the pattern of the response. Reduction in the post-prandial values in healthy subjects was significant at the 1% level of probability, while reductions in the fasting and post-prandial fatty acid concentrations in atherosclerotic subjects attained the 5% and 1% levels of significance respectively.

It is also clear that the height and shape of the fatty acid response differed in the two groups of subjects. The concentration of total fatty acids in the fasting state was significantly lower among the healthy subjects when saturated fat diets were being consumed ($P<0·01$), while the post-prandial levels in these subjects were lower than in atherosclerotic subjects during both dietary regimens ($P<0·001$). In addition, the fatty acid curves in atherosclerotic subjects appeared to rise more steeply to greater heights above their origins than in the healthy subjects, while the decline towards the fasting levels at seven hours was not so obvious.

Serum Phospholipid Content

The phospholipid content of serum rose steadily after ingestion of the test meal. The increase, although not marked, was statistically significant (Table III) under all circumstances, with the exception of the healthy subjects during the period of sunflower-seed oil substitution. Failure to reach significance in this case can be attributed to the small number of observations, and to their rather wide scatter.

Figure IV suggests that the presence or absence of atherosclerosis had little influence on the shape or the height of the phospholipid curves. Comparisons between the groups of subjects failed to demonstrate any differences either of the fasting or of the post-prandial averages of phospholipid concentration shown in Table III.

The use of unsaturated fat failed to induce any marked change in the shape of the phospholipid responses, although it set the curves at lower levels. Analysis showed that sunflower-seed oil produced falls in the fasting phospholipid content, significant at the 5% level of probability in healthy subjects and at the 1% level in atherosclerotics. Although the phospholipid levels rose after the test meal, they remained significantly lower ($P<0·01$) than the levels attained after the test meal in the control period.

The Serum Cholesterol Concentration

Ingestion of the fat meal had no significant effect on the serum cholesterol concentration (Table III) in either group of subjects.

In Figure IV it can be seen that sunflower-seed oil lowered the fasting and post-prandial

TABLE III

The Averages for the Fasting and Pooled Post-Prandial Values of the Plasma Optical Density Recordings and Serum Lipid Concentrations, Together with the Standard Deviations and the Significances of the Differences

Characteristic	Saturated Fat Diet						Unsaturated Fat Diet					
	Healthy Subjects (15 Observations)			Atherosclerotic Subjects (17 Observations)			Healthy Subjects (8 Observations)			Atherosclerotic Subjects (9 Observations)		
	Fasting	Pooled Post-Prandial Data	Significance of the Difference	Fasting	Pooled Post-Prandial Data	Significance of the Difference	Fasting	Pooled Post-Prandial Data	Significance of the Difference	Fasting	Pooled Post-prandial Data	Significance of the Difference
Optical density of plasma ..	111·93±16·15	236·78±117·99	$P<0·001$	94·71±10·55	247·54±122·07	$P<0·001$	125·25±31·59	233·72±93·70	$P<0·01$	93·56±16·08	274·83±162·70	$P<0·01$
Serum total fatty acid content (mEq. l) ..	11·13±1·41	13·78±2·09	$P<0·001$	13·73±2·48	18·09±4·23	$P<0·001$	10·06±1·45	12·29±2·61	$P<0·05$	11·63±1·64	15·67±3·72	$P<0·01$
Serum phospholipid content (mg. per 100 ml.)	230·4 ±26·49	265·22±25·44	$P<0·05$	249·47±27·48	269·41±30·43	$P<0·02$	214·88±35·78	234·22±33·44	$P>0·05$	219·11±19·62	243·19±22·67	$P<0·01$
Serum total cholesterol content (mg. per 100 ml.)	232·4 ±18·90	240·22±23·14	$P>0·05$	252·12±33·03	255·06±31·57	$P>0·05$	192·25±22·30	195·97±23·36	$P>0·05$	210·11±22·24	221·53±24·33	$P>0·05$

cholesterol concentrations in both groups of subjects. Reduction of the fasting serum cholesterol concentration reached the 0·1% level of probability in healthy subjects and the 2% level of significance in atherosclerotic subjects, while reductions in the post-prandial values were significant at the 0·1% level in both groups.

It is also clear that the presence of atherosclerosis was associated with higher serum cholesterol levels throughout the observation period. Comparisons between the subject groups revealed that the fasting serum cholesterol level was lower in healthy subjects during both dietary periods ($P<0·05$), and that the post-prandial concentrations were also lower during the consumption of both the saturated ($P<0·01$), and the unsaturated ($P<0·001$) fat diets.

Relationships between the Serum Lipid Concentrations and Measures of Blood Clotting

In order to confirm and perhaps extend the observations already made, correlation coefficients for lipid-lipid, lipid-clotting and clotting-clotting relationships were calculated. These have been derived separately for each group of subjects from data pooled from the fat tolerance tests carried out during both dietary periods.

Healthy Subjects.—In Table IV it can be seen that there is no relationship between the "Stypven" time and the W.B.C.T. during the course of alimentary lipæmia. This is in accord with other observations made solely in the fasting state (Goldrick and Whyte, 1959; Goldrick, 1960). A likely explanation comes from the lipid-clotting associations shown in Table IV. There it will be observed that the W.B.C.T. has a negative and statistically significant association with the serum phospholipid level, but no relation to total cholesterol content, or to plasma turbidity and total fatty acid content, which are indirect measures of the chylomicron population. On the other hand, the "Stypven" time has a definite negative relationship with plasma turbidity and fatty acid level, but no relation with the phospholipid or cholesterol levels. In other words, acceleration of the W.B.C.T. is associated with a rise in phospholipid concentration, while acceleration of clotting in the "Stypven" test is associated with an increase in chylomicron fatty acids. These measures of blood clotting are presumably influenced by different serum lipids, and although both change after the ingestion of fat, the changes do not run parallel.

From a consideration of the correlation coefficients in Table V it is clear that the

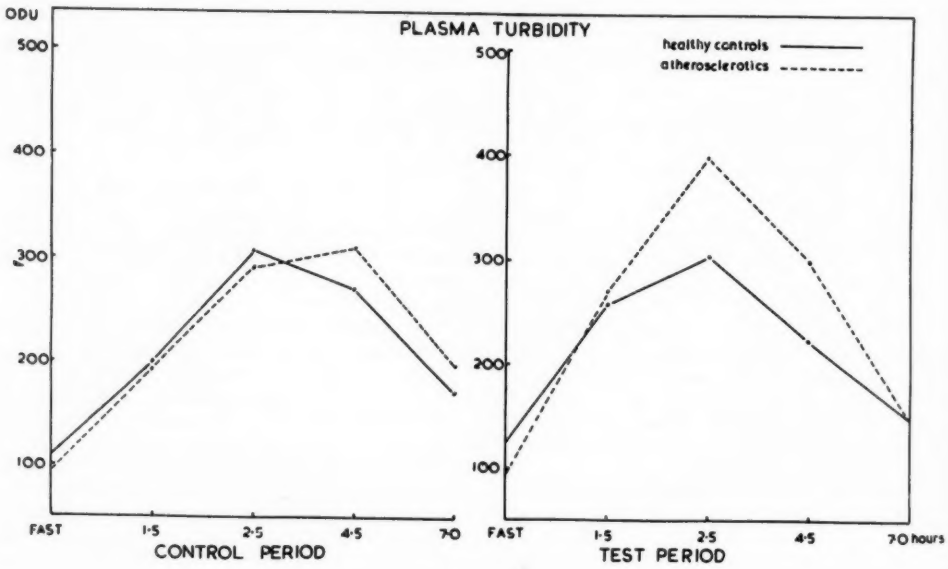

FIGURE III
The influence of dietary fats on the plasma turbidity responses to a test meal of cream

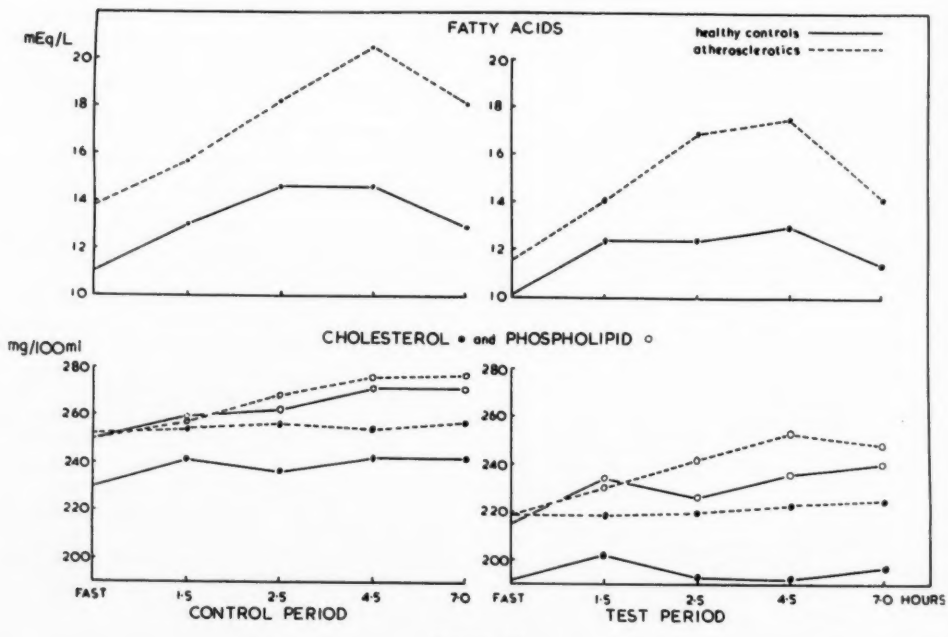

FIGURE IV
The influence of dietary fats on the serum lipid responses to a test meal of cream

TABLE IV

Correlation Coefficients (with Levels of Significance) for the Serum Lipids and Measures of Blood Clotting in Healthy Young Men; 115 Observations

—	"Stypven" Time	Plasma Density	Fatty Acid Content	Phospholipid Content	Cholesterol Content
W.B.C.T.	−0·040⁻	−0·135⁻	−0·003⁻	−0·357***	−0·094⁻
"Stypven" time		−0·617***	−0·583***	−0·104⁻	−0·122⁻
Plasma density			+0·508***	+0·014⁻	−0·020⁻
Serum fatty acid content				+0·314***	+0·268**
Serum phospholipid content					+0·823**

*, ** and *** denote P values less than 0·05, 0·01 and 0·001 respectively; "⁻" denotes insignificance.

fasting W.B.C.T. has an important influence on its subsequent response to the test meal. The coefficients relating the fasting W.B.C.T. to its percentage maximum change, to its maximum absolute change and to the difference between the fasting clotting time and the clotting time at seven hours, are positive and statistically significant. An explanation for these relationships is offered on the basis of the following hypothesis. It is assumed that, so far as the influence of at least some of the blood lipids is concerned, there are both upper and lower limits for the clotting time as measured in this test. If the W.B.C.T. is at its upper limit of normal when the fat tolerance test is begun, then the fatty meal exerts a maximum accelerating effect on clotting, thereby inducing a large percentage maximum change, a large absolute maximum change and a prolonged effect. However, should the fasting W.B.C.T. already be short, ingestion of fat will induce only a limited change.

The foregoing hypothesis also provides an explanation for the significant and negative associations in Table V between the fasting serum cholesterol and phospholipid levels and the magnitude of the post-prandial changes in the W.B.C.T. Significant and negative relationships exist between the serum cholesterol level, the serum phospholipid level and the W.B.C.T. in the fasting state (Goldrick, 1960). It follows that a rise in the serum phospholipid and/or cholesterol level in the fasting state is associated with a shortening of the W.B.C.T. A fatty meal given under these circumstances could effect only small changes in clotting.

There is little evidence in Table V to suggest that the initial rate of clotting in the "Stypven" test or the levels of the serum lipids measured in the fasting state have any influence on the magnitude of the response of the "Stypven" test to the fat meal.

Atherosclerotic Subjects.—In atherosclerotic subjects, the W.B.C.T. is found to vary significantly and in the same direction as the rate of clotting in the "Stypven" test (Table VI). This finding is in accord with other observations made in the fasting state in atherosclerotics (Goldrick, 1960).

The correlation coefficients in Table VI are considered to imply that the optical density of plasma, and the serum cholesterol, phospholipid and fatty acid levels rise and fall together after ingestion of the test meal, maintaining approximately similar proportions one to the

TABLE V

Correlation Coefficients for the Fasting Characteristics of the Fat Tolerance Test, and Percentage and Absolute Maximum Changes and the Changes at Seven Hours in the Clotting Tests among Healthy Men; 23 Observations

Fasting Characteristic	Percentage Maximum Change		Maximum Absolute Change		Change at Seven Hours	
	W.B.C.T.	"Stypven" Time	W.B.C.T.	"Stypven" Time	W.B.C.T.	"Stypven" Time
W.B.C.T.	+0·673***	−0·122⁻	+0·830***	−0·201⁻	+0·598***	+0·473*
"Stypven" time	+0·038⁻	−0·333⁻	−0·005⁻	+0·211⁻	+0·262⁻	+0·018⁻
Plasma density	−0·224⁻	+0·066⁻	−0·202⁻	−0·202⁻	−0·380⁻	−0·274⁻
Serum fatty acid content	+0·085⁻	+0·091⁻	+0·016⁻	−0·061⁻	−0·139⁻	+0·240⁻
Serum phospholipid content	−0·604***	+0·149⁻	−0·662***	+0·261⁻	−0·636***	−0·322⁻
Serum cholesterol content	−0·469*	+0·242⁻	−0·450*	+0·229⁻	−0·483*	−0·099⁻

*, ** and *** denote P values less than 0·05, 0·01 and 0·001 respectively; "⁻" denotes insignificance.

TABLE VI

Correlation Coefficients (with Levels of Significance) for the Serum Lipids and Measures of Blood Clotting in Atherosclerotic Men Fasting and During Alimentary Lipæmia; 130 Observations

	"Stypven" Time	Plasma Density	Fatty Acid Content	Phospholipid Content	Cholesterol Content
W.B.C.T.	+0·373***	−0·371***	−0·403***	−0·400***	−0·297**
"Stypven" time		−0·553***	−0·553***	−0·391***	−0·263**
Plasma density			+0·695***	+0·433***	+0·252*
Fatty acid content				+0·814***	+0·602***
Phospholipid content					+0·817***

*, ** and *** denote P values less than 0·05, 0·01 and 0·001 respectively; "−" denotes insignificance.

other throughout the observation period. Significantly associated with these changes are inverse alterations in the measures of blood clotting (see Figure I).

From inspection of Table VII, it is clear that the magnitude of the response of the W.B.C.T. to the test meal in these subjects is also dependent on the fasting W.B.C.T. Moreover, the significant and negative relationships between the fasting serum cholesterol level and the percentage maximum change, and between the fasting fatty acid level and the maximum absolute change and the difference at seven hours in the W.B.C.T., reflect the inverse relationship which exists between these lipids and the W.B.C.T. (Goldrick, 1960).

The longer post-prandial W.B.C.T. during the exhibition of sunflower-seed oil probably depended on the longer fasting clotting times. Evidently the test meal produced a considerable acceleration of clotting when the fasting clotting times were long, but its effect was insufficient to produce clotting times as short as those observed when the subjects were on a saturated fat diet. Had a larger dose of cream been administered, it is likely that the post-prandial W.B.C.T. would have been similar in both dietary periods.

The significant association between the fasting "Stypven" time and its maximum post-prandial change (Table VII) indicates that the result of this test in atherosclerotics tends to behave in a similar fashion to that of the W.B.C.T. In view of this fact, and of the close relationship between the "Stypven" time and the W.B.C.T. in these subjects, it is surprising to find no relation between the fasting lipid levels and the magnitude of the "Stypven" response.

DISCUSSION

It is generally agreed that the "Stypven" time undergoes an acceleration during the course of alimentary lipæmia (Fullerton *et alii*, 1953; O'Brien, 1955; Maclagan *et alii*, 1958), and the results of this study are no exception to this rule. However, there is no unanimity of opinion as to the effect of a fatty meal on the W.B.C.T. Fullerton *et alii* (1953), Waldron and Nichols (1952), Buzina and Keys (1956), Mandel and Francois (1957) and O'Brien (1955) reported a shortening of the W.B.C.T. after meals of fat, while Manning and Walford (1954), Sohar *et alii* (1956), Merskey and Nossel (1957) and Borrero *et alii* (1958) were unable to demonstrate any consistent change. The data

TABLE VII

Correlation Coefficients for the Fasting Characteristics of the Fat Tolerance Test and Percentage and Absolute Maximum Changes and the Changes at Seven Hours in the Clotting Tests among Atherosclerotic Subjects; 26 Observations

Fasting Characteristic	Percentage Maximum Change		Maximum Absolute Change		Change at Seven Hours	
	W.B.C.T.	"Stypven" Time	W.B.C.T.	"Stypven" Time	W.B.C.T.	"Stypven" Time
W.B.C.T.	+0·737***	−0·272⁻	+0·875***	−0·176⁻	+0·694***	−0·331⁻
"Stypven" time	+0·158⁻	+0·105⁻	+0·127⁻	+0·576**	+0·061⁻	+0·188⁻
Plasma density	+0·002⁻	+0·147⁻	−0·064⁻	+0·006⁻	−0·246⁻	−0·146⁻
Serum fatty acid content ..	−0·355⁻	+0·005⁻	−0·392*	−0·101⁻	−0·436*	−0·211⁻
Serum phospholipid content	−0·198⁻	+0·076⁻	−0·237⁻	−0·046⁻	−0·332⁻	−0·260⁻
Serum cholesterol content ..	−0·735***	+0·248⁻	−0·269⁻	+0·064⁻	−0·352⁻	+0·005⁻

*, ** and *** denote P values less than 0·05, 0·01 and 0·001 respectively; "−" denotes insignificance.

presented here leave no doubt that a shortening of the W.B.C.T. does indeed occur, but provide no explanation for the differences of opinion already referred to.

In the writer's hands the "Stypven" test has proved reliable, reproducible and easy to carry out, while the W.B.C.T. can vary greatly unless strict precautions are taken. These include proper preparation of the subject and care in the technique of the test. The latter has been fully described elsewhere (Goldrick and Whyte, 1959), and it is only necessary to emphasize that the venepuncture must be absolutely "clean", no probing under the skin being acceptable. As far as the subject is concerned, it is essential that he be in the basal state. Physical discomfort, such as a distended bladder, disagreements with the nursing staff, a disturbed night's sleep or anxiety, usually resulted in a fasting clotting time shorter than that usually found in that subject. For these reasons, it was usually desirable to delay the first fat tolerance test until the procedure had been explained and a venepuncture previously performed, so that the subject's confidence could be obtained. These opinions echo Waldron and Duncan's (1954) observations that the subject must be calm, comfortable and at rest if the W.B.C.T. is to be reliable. On those occasions when the subject was not in the basal state, the fasting clotting time was often as short as, or shorter than, that of the later lipæmic samples. Perhaps one reason for the present confusion in regard to the W.B.C.T. response to alimentary lipæmia has been a failure to appreciate how sensitive is this test to extraneous factors.

In accordance with the suggestion that blood coagulation may be a factor in atherogenesis (Fullerton *et alii*, 1953; Duguid, 1954), several studies have been made to determine whether there exists any relationship between the coagulation response to alimentary lipæmia and the incidence of atherosclerotic complications. O'Brien (1958) was unable to demonstrate any difference between healthy controls and atherosclerotic subjects in regard to fasting clotting times or clotting times measured four hours after a fatty meal, while Mustard (1958) found blood clotting to be somewhat shorter in atherosclerotic subjects than in healthy people during the course of alimentary lipæmia. The results of the present, albeit limited, study indicate that under ordinary circumstances the blood clotting responses are similar. Although this subject requires further investigation, it is clear that if a difference does exist between the healthy subject and the atherosclerotic, then it must be a small one and subject to a good deal of overlap between groups.

When blood clotting and serum lipid concentrations were studied in the fasting state (Goldrick, 1960) it was shown that reduction in the serum lipid concentrations was without effect on the "Stypven" time in atherosclerotic and healthy subjects, and on the W.B.C.T. in the controls, but in the atherosclerotic subjects a lengthening of the W.B.C.T. occurred. These features noted in the fasting state were also observed during alimentary lipæmia, the W.B.C.T. in atherosclerotics being longer both before and after the test meal when the fasting serum lipid levels were low. Dietary changes produced no alteration in the way in which the "Stypven" test in both groups and the W.B.C.T. in healthy men responded to alimentary lipæmia. Reasons have been given earlier for concluding that the longer postprandial W.B.C.T. in atherosclerotic subjects observed when the fasting serum lipid levels were low were merely reflecting the lengthening that had occurred in the fasting W.B.C.T.

These observations in atherosclerotic subjects are of considerable interest, but they are by no means conclusive. If dietary manipulations were not only to lower the serum lipid levels, but also to produce a lengthening of the W.B.C.T. and a change in its response to fatty meals, a twofold purpose would be served: less lipid would filter into the intima and less fibrin would be deposited there; presumably the pace of the atherosclerotic process and its thrombotic complications would be slowed. However, the number of subjects is small, and they may not be representative of the atherosclerotic population in general. In addition, it is not known whether this inhibition of clotting would continue for as long as the serum lipids were maintained at low concentrations, or whether in time it would diminish and allow the W.B.C.T. to return to its original level. Nevertheless, when one thinks along the lines of the lipid-infiltration and thrombotic theories for the pathogenesis of vascular disease, the present study does provide evidence which suggests that lowering of the serum lipid levels—for example, by consuming unsaturated fats—might be of benefit to those suffering from ischæmic heart disease and related vascular disorders.

It has been reported that atherosclerosis is associated with a high optical density of plasma both in the fasting state and during alimentary lipæmia (Barritt, 1956), while a similar difference in regard to chylomicron counts has been associated with increasing age (Becker *et alii*, 1949). Since chylomicrons are composed for

the most part of triglycerides (Bragdon *et alii*, 1956) and the turbidity of plasma after a fatty meal is due to their presence, the total fatty acid and optical density measurements are indirect measures of particulate fat. For these reasons, it is surprising that no differences in plasma turbidity could be demonstrated between the young controls and older atherosclerotic subjects, either before or during the fat tolerance tests. On the other hand, the total fatty acid levels in the fasting state were higher in the atherosclerotic group, and appeared to increase rather more than in the younger men and to remain elevated at higher levels above the fasting measurements at the end of seven hours. Why there should be a discrepancy between these two measurements is uncertain; possibly the absorption measurements of plasma at 420 mμ were influenced by other factors besides the number of chylomicrons. It is worthy of note that the phospholipid levels in the fasting and lipæmic states were similar in both groups, while the differences in cholesterol concentrations in the fasting state were maintained throughout the course of the tolerance tests.

Attempts have been made by several investigators to relate the clotting changes occurring during alimentary lipæmia to the levels of particulate and non-particulate fat. For the most part, these have been unrewarding or have provided contradictory results, and indeed it is difficult to compare the results of one worker with those of another, owing to differences in the test meals used, in the age and type of subjects, in the accuracy or otherwise of the methods, and in the timing and the number of samples measured during lipæmia. Keys *et alii* (1957) found no relationship between visible lipæmia and acceleration of the W.B.C.T., nor could O'Brien (1955) demonstrate any relationships between particulate and non-particulate fat and the changes in the W.B.C.T., in the result of the thrombin generation test, and in the "Stypven" time. Later, O'Brien (1957) found no correlation between the changes in the "Stypven" time and the total fatty acid levels, and only poor evidence that considerable changes in the results of this clotting test were associated with an increase in total phospholipid concentrations. However, Fullerton *et alii* (1953) noted that shortening of the W.B.C.T. occurred only when macroscopic lipæmia developed. Sohar *et alii* (1956) reported a relationship between neutral fats and shortening of the "Stypven" time, and Maclagan *et alii* (1958) found a relationship between the increase in plasma turbidity and acceleration of the "Stypven" time.

In view of the rather contradictory evidence that the ordinary measures of particulate and non-particulate fat have any relation to the post-prandial changes in blood coagulation, and particularly when the evidence from a large number of studies *in vitro* is considered (*vide infra*), a discussion of further statistical associations in this regard might appear unproductive. However, it seems hardly fortuitous that the correlation coefficients in Tables IV and VI should bear such a striking similarity to the lipid-clotting and clotting-clotting associations already described in the fasting state (Goldrick, 1960). For this reason, some attempt at explanation is warranted.

The correlation coefficients in Table IV imply that as total phospholipid levels increase in the healthy men the W.B.C.T. becomes shorter, while the "Stypven" time accelerates as the concentration of particulate fat rises; however, the result of neither clotting test bears any relation to the other. In the atherosclerotic group, on the other hand, the results of the clotting tests are significantly related to one another, and are shortened as the concentrations of all the lipid fractions, including chylomicrons, increase. However, studies *in vitro* (Rouser *et alii*, 1958) indicate that cholesterol, triglycerides, amino acids and most phospholipids are without effect on the plasma recalcification time and "Stypven" time, but phosphatidyl ethanolamine (P.E.) has a pronounced coagulant action in extremely small concentrations, and long-chain unesterified fatty acids are slightly coagulant. Chylomicrons have coagulant effects on plasma clotting *in vitro* (Poole, 1955), and owe this effect to the presence of small amounts of P.E. or P.E.-like compounds (Poole and Robinson, 1956; Robinson and Poole, 1956). In addition, the concentration of unesterified fatty acids is said to increase during alimentary lipæmia (Robinson *et alii*, 1955). Presumably, then, the associations in Tables IV and VI are reflecting, in an indirect fashion, changes in chylomicron P.E. and/or unesterified fatty acids. This is conceivable because the post-prandial rise in blood fat levels is mainly due to chylomicrons. Why, then, are the lipid-clotting and clotting-clotting associations different among the two groups of subjects? One possible, but rather unlikely, explanation is that healthy people and atherosclerotic subjects differ in the way in which their blood coagulability is affected by P.E. and/or unesterified fatty acids. A much more likely explanation is that the W.B.C.T. is influenced mainly by unesterified fatty acids, and the "Stypven" test result by P.E. (or *vice versa*). If the second hypothesis is correct, it follows that P.E. and unesterified

fatty acids must vary together in the older atherosclerotic subjects in order that the "Stypven" time and the W.B.C.T should be related, while P.E. and unesterified fatty acids must vary independently in healthy young people to account for the lack of association between the clotting times in these subjects.

The correlations already discussed also point to the possible role of the fasting lipoproteins in blood coagulation. It has been noted that the serum concentrations of lipoproteins and of chylomicrons (indicated by the levels of their constituent lipids) possess statistically significant and qualitatively similar relationships with the rate of blood clotting. Moreover, chylomicrons exhibit coagulant properties *in vitro*, owing to their content of small amounts of highly active phosphatides. Perhaps, then, it is not too fanciful to predict that the lipoproteins of fasting serum will also be found to contain these phosphatides and exhibit coagulant properties *in vitro*.

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SERUM LIPOPROTEINS AND BLOOD COAGULATION IN HEALTHY AND ATHEROSCLEROTIC MEN[1]

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SUMMARY

Serial estimations of serum lipids and blood clotting were made under basal conditions in nine healthy young men and in 10 atherosclerotic older men.

When the serum concentrations of beta lipoprotein lipids were reduced by substituting unsaturated fats in the diet, there was a significant lengthening of the whole blood clotting time (W.B.C.T.) in the atherosclerotic subjects. No such change occurred in the healthy controls, and the "Stypven" time was unaltered in both groups of subjects.

The "Stypven" time was consistently longer in the healthy men, but there was no difference in the W.B.C.T. between subjects until clotting in the atherosclerotic subjects became longer with the fall in serum lipid levels.

Certain differences in the lipoprotein transport of cholesterol were noted. In the healthy subjects a rise in total cholesterol level was borne by both alpha and beta lipoproteins, although the beta fraction was mainly responsible for the rise in total cholesterol level. On the other hand, in the atherosclerotic subjects a rise in total cholesterol level was associated with a fall in alpha and a rise in beta lipoprotein. The significance of these differences in regard to atherogenesis is discussed.

Among the healthy subjects alpha lipoprotein was associated with longer clotting times in the "Stypven" test and shorter W.B.C.T. On the other hand, in the atherosclerotic men both measures of clotting varied together. As the concentration of alpha lipoprotein increased, clotting times became longer, and as the beta lipoprotein concentration rose, clotting was accelerated. These associations suggest that the serum lipoproteins or some closely associated factors influence the rate of blood coagulation.

A GOOD deal of indirect evidence—epidemiological, experimental, clinical and biochemical—suggests that the amount and perhaps the nature of the dietary fat, by affecting the concentrations of the serum lipids, is an important factor in atherogenesis. According to this view, the atherosclerotic plaque is an end-result of lipid infiltration, while thrombus formation occurs secondarily because of vascular narrowing and roughening. On the other hand, there is equally suggestive evidence that thrombus formation is the primary factor, while the fat which is found in the plaque is the residue of degenerated formed elements originally present in the clot.

If the concentrations of the serum lipids were to influence the rate of blood clotting and fibrin deposition, there would be truth in both hypotheses. A lipoid factor seems necessary for blood to clot in a normal fashion (Macfarlane *et alii*, 1941), and blood coagulation is accelerated during the course of alimentary lipæmia (Fullerton *et alii*, 1953); but there is only limited evidence to suggest that the concentration of fats in fasting blood plays an important part in regulating coagulability. McDonald and Edgill (1958) reported a decrease in platelet stickiness, but no changes in several other measures of blood clotting, when the serum cholesterol concentration was lowered by a diet of rice and fruit, while studies in healthy Australians and natives of New Guinea demonstrated a statistically significant association between alpha lipoprotein and clotting in the "Stypven" test (Goldrick and Whyte, 1959).

In the present study the serum lipid levels were lowered by isocaloric substitution of unsaturated fats (sunflower-seed oil) for saturated fats in the diet, and blood clotting was measured at weekly intervals under basal conditions. The object was to determine whether a change in blood clotting would accompany alteration in the serum lipid levels, and whether the changes in coagulability (if any) were different in healthy and atherosclerotic men. In addition, it was hoped to confirm and perhaps further elucidate the association between alpha protein and clotting in the "Stypven" test.

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[2] Research Fellow, supported by an Anonymous Family Trust.

The previous study had suggested that an increased concentration of alpha lipoprotein inhibited clotting, but this was derived from observations made among a number of subjects; it had yet to be demonstrated that a similar relationship existed for any one individual when his serum lipoprotein concentration was forced to change.

MATERIALS AND METHODS

Subjects

The atherosclerotic group comprised 10 men whose ages ranged from 44 to 64 years, averaging 50 years. They all presented with unequivocal evidence of the complications of atherosclerosis. The diagnosis in four cases was angina pectoris; in one case it was peripheral vascular disease, angina pectoris and an old myocardial infarction; and in another, it was angina pectoris and an old myocardial infarction. Of the remaining patients, three were admitted to hospital with mild myocardial infarctions, and one was hemiplegic from a cerebral vascular thrombosis. All patients were investigated to exclude the presence of liver disease, acute infections and diabetes mellitus, and to provide supportive evidence for the diagnosis of atherosclerotic complications. None had received anti-coagulant therapy.

Subjects for the control group were drawn from the resident medical staff of Sydney Hospital. They were nine healthy men whose ages ranged from 23 to 33 years, averaging 26 years.

Diet

At the beginning of the study, each subject was interviewed by the dietitian and the caloric contributions from fat, carbohydrate and protein in his customary diet were calculated.

Control Period

For the first three weeks of observation, each subject consumed his usual diet, with the exception of those individuals whose physical activities had recently been restricted. In those cases the total caloric intake was adjusted to individual requirements, while the percentages of total calories derived from fat, carbohydrate and protein were kept constant. By this means variations in serum lipid levels were limited.

Test Period

During the following four weeks, sunflower-seed oil was substituted isocalorically for saturated fat in the diet. The protein and carbohydrate intake remained unchanged. On the first day, 50 grammes of sunflower-seed oil were administered, and then the daily dose was gradually increased until, by the end of the first week, 80 to 100 grammes were being consumed. Thereafter the intake of oil was constant. Attempts to eat 80 to 100 grammes of oil without a preliminary induction period usually resulted in severe gastro-intestinal intolerance—nausea, vomiting and occasionally diarrhœa—while gradual introduction of the oil was uneventful. The amount of oil administered was determined by the number of calories supplied by dietary fat in the control period, 25 grammes per day being the practical minimum to which saturated fats could be reduced without recourse to synthetic diets; the dose of sunflower-seed oil thus equalled total fat consumed minus 25 grammes.

The oil was given in the form of a mixture, 50 to 100 grammes of sunflower-seed oil being blended with 118 grammes of fat-free evaporated milk powder in 500 ml. of water, flavoured according to the subject's taste, and chilled. The remaining calories were derived from conventional foods.

The subjects were weighed weekly, and body weight was maintained constant by varying carbohydrate consumption when necessary.

Serum Lipid and Blood Clotting Determinations

At the beginning of each week, under basal conditions, blood was collected by clean venepuncture with siliconed apparatus. The measurements performed included the whole blood clotting time in siliconed tubes (W.B.C.T.), the "Stypven" clotting time and the serum total cholesterol content, phospholipid content, total esterified fatty acid content and alpha and beta lipoprotein cholesterol content (paper electrophoresis and a cholesterol elution). The methods, for the most part, have been described elsewhere (Goldrick and Whyte, 1959). The total esterified fatty acid content was determined by the method of Stern and Shapiro (1953); the standard deviation of the differences between duplicates was 0·69 mEq./l of serum derived from the expression

$$\sigma_e = \sqrt{\frac{\Sigma d^2}{2n}}.$$

RESULTS

The average values of the various characteristics measured (together with the significances of the differences) in each dietary period and for both groups of subjects are given in Table I. The weekly averages for the healthy controls are illustrated in Figure I and for the atherosclerotic subjects in Figure II.

TABLE I

Average Values for the Serum Lipids and Clotting Times (with Standard Deviations) for Nine Healthy Young Men and Ten Atherosclerotic Middle-Aged Men when Consuming their Usual Diets, and Diets Containing Sunflower-Seed Oil

Characteristic	Control Diet		With Sunflower-Seed Oil		Significance of the Differences			
					Control *v.* S.S. Oil		Normals (Men) *v.* Atherosclerotic Men	
	Normal Men	Atherosclerotic Men	Normal Men	Atherosclerotic Men	Normal Men	Atherosclerotic Men	Control	S.S. Oil
Serum cholesterol concentration (mg. per 100 ml.) ..	235·6±21·54	261·4±46·84	195·5±19·93	226·8±32·83	<0·001	<0·001	<0·02	<0·001
Serum fatty acid concentration (mEq./l)	11·7±1·61	17·2±10·30	10·2±1·61	13·7±7·45	<0·001	—	<0·01	<0·01
Serum phospholipid concentration (mg. per 100 ml.)	254·8±21·65	267·9±64·0	220·6±24·19	231·1±36·19	<0·001	<0·01	—	—
Serum alpha cholesterol concentration (mg. per 100 ml.)	49·0±13·12	42·8±12·90	43·5±12·29	38·3±10·87	—	—	<0·01	
Serum beta cholesterol concentration (mg. per 100 ml.)	186·9±21·50	218·6±54·42	152·2±20·33	188·3±38·72	<0·001	<0·01	<0·01	<0·001
W.B.C.T. (minutes) ..	27·5±4·67	27·4±3·32	27·1±4·31	30·8±6·44	—	<0·02	—	<0·01
"Stypven" clotting time (seconds)	70·2±14·18	63·6±11·31	68·4±14·04	64·7±12·81	—	—	<0·05	

The Serum Lipid Responses to Unsaturated Fats

Healthy Controls.—The serum cholesterol concentration remained relatively constant during the initial period of observation, averaging 235·6 mg. per 100 ml. A prompt and statistically significant fall in the serum cholesterol concentration followed substitution of sunflower-seed oil in the diet, and was maximal by the end of the second week. The average serum cholesterol concentration throughout the sunflower-seed oil period was 195·5 mg. per 109 ml.

The phospholipid content of serum closely followed the changes in serum cholesterol concentration. Dietary change-over resulted in a significant reduction in phospholipid concentration from 254·8 to 220·6 mg. per 100 ml.

A small but statistically significant fall in fatty acid concentration (1·5 mEq./l) was also noted during the consumption of unsaturated fats.

Dietary manipulation resulted in a significant lowering of beta lipoprotein cholesterol concentration from 186·9 to 152·2 mg. per 100 ml., while the concentration of alpha lipoprotein cholesterol was unchanged.

In Figure I it is clear that the serum concentrations of cholesterol, phospholipids, fatty acids and beta lipoprotein cholesterol varied together. When the constant level of alpha lipoprotein is taken into account, the changes produced in the serum cholesterol, fatty acid and phospholipid concentrations were merely reflecting changes in the parent beta lipoprotein concentration.

Atherosclerotic Subjects.—The serum lipid responses to unsaturated fats in these subjects were similar to those observed in the healthy controls. A rapid and statistically significant fall in the serum concentration of cholesterol followed the exhibition of sunflower-seed oil, the reduction becoming maximal by the end of the second week. The serum cholesterol concentration averaged 261·4 mg. per 100 ml. during the control period and 226·8 mg. per 100 ml. during the latter four weeks of observation.

There was also a statistically significant fall in the serum concentration of phospholipids, from an average of 267·9 mg. per 100 ml. in the control period to 231·1 mg. per 100 ml. when unsaturated fats were being consumed. The phospholipid changes ran parallel to the variations in total cholesterol concentration.

It is clear that the concentrations of phospholipids and cholesterol were almost identical in the atherosclerotic subjects (Figure II), indicating a P-C ratio near unity, whereas in the healthy controls the phospholipid levels were consistently higher than those of cholesterol (Fig. I), the P-C ratio being greater than one.

In spite of a relatively large fall in the concentration of fatty acids from 17·2 to 13·7 mEq./l, the difference was not statistically significant. This is probably accounted for by the wide fluctuations in fatty acids in this group.

The dietary change produced a significant reduction in beta lipoprotein cholesterol concentration from 218·6 mg. per 100 ml. in the control period to 188·3 mg. per 100 ml., while the alpha lipoprotein cholesterol concentration was unchanged.

In these subjects, also, variations in the serum concentrations of cholesterol phospholipids and fatty acids were accounted for by changes in beta lipoprotein concentration. The weekly average concentrations of these lipids (Figure II) ran closely parallel to each other, while that of alpha lipoprotein cholesterol remained constant throughout.

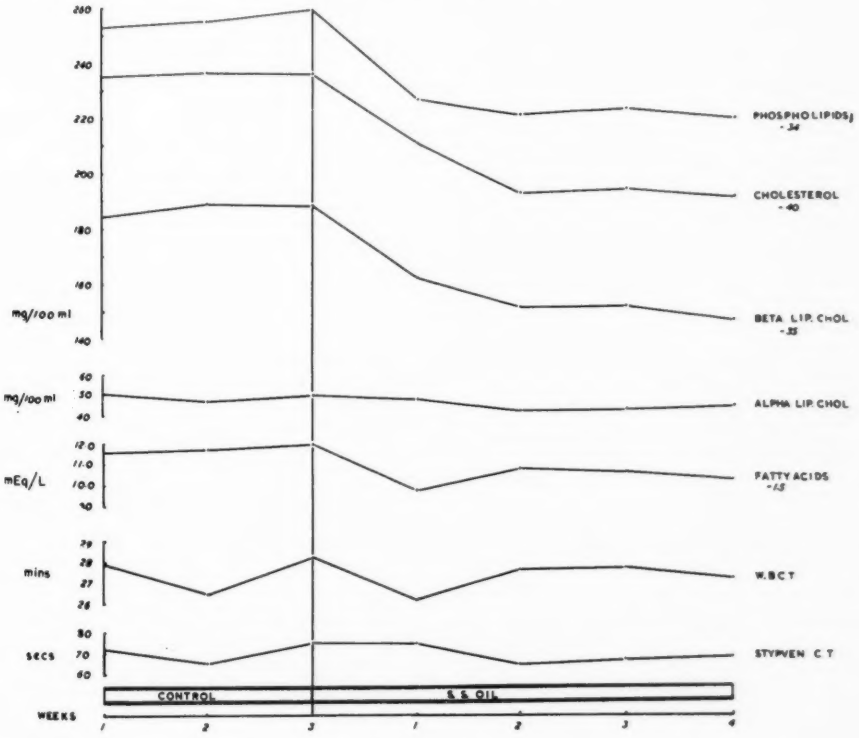

FIGURE I

The effects of sunflower-seed oil on the serum lipids and measures of blood clotting in healthy young men

Comparison of the Serum Lipid Levels between the Healthy Controls and the Atherosclerotic Subjects

The serum concentrations of total cholesterol, fatty acids and beta lipoprotein cholesterol were consistently lower in the healthy subjects than in the atherosclerotic subjects during both periods of observation, while the phospholipid levels were similar.

No significant difference between the groups in regard to alpha lipoprotein cholesterol concentration could be demonstrated until data from both the dietary periods were pooled. It was then apparent that the concentration of alpha lipoprotein cholesterol in the healthy controls (45·9 mg. per 100 ml.) was significantly higher than in the atherosclerotic subjects (40·2 mg. per 100 ml.; $P<0·01$).

The Effects of Diet on Blood Clotting

Healthy Controls.—Alterations in diet produced no change in the W.B.C.T. This averaged 27·5 minutes during the control period and 27·1 minutes during the sunflower-seed oil period. The "Stypven" time was also unaltered, averaging 70·2 seconds in the control period and 68·4 seconds after the dietary change-over.

Atherosclerotic Subjects.—The W.B.C.T. became progressively longer when unsaturated

fats were consumed and averaged 30·8 minutes. The difference between this value and that found during the taking of ordinary diets (27·4 minutes) was statistically significant. There was no change in the "Stypven" time. It averaged 63·6 seconds in the initial period of observation and 64·7 seconds when sunflower-seed oil was substituted.

Relationships between the Various Lipid Concentrations, between the Measures of Blood Clotting and between the Lipid Concentrations and Measures of Blood Clotting

The correlation coefficients for lipid-lipid, clotting-clotting and lipid-clotting relationships have been calculated. They have been derived separately for each group of subjects from data pooled from both dietary periods.

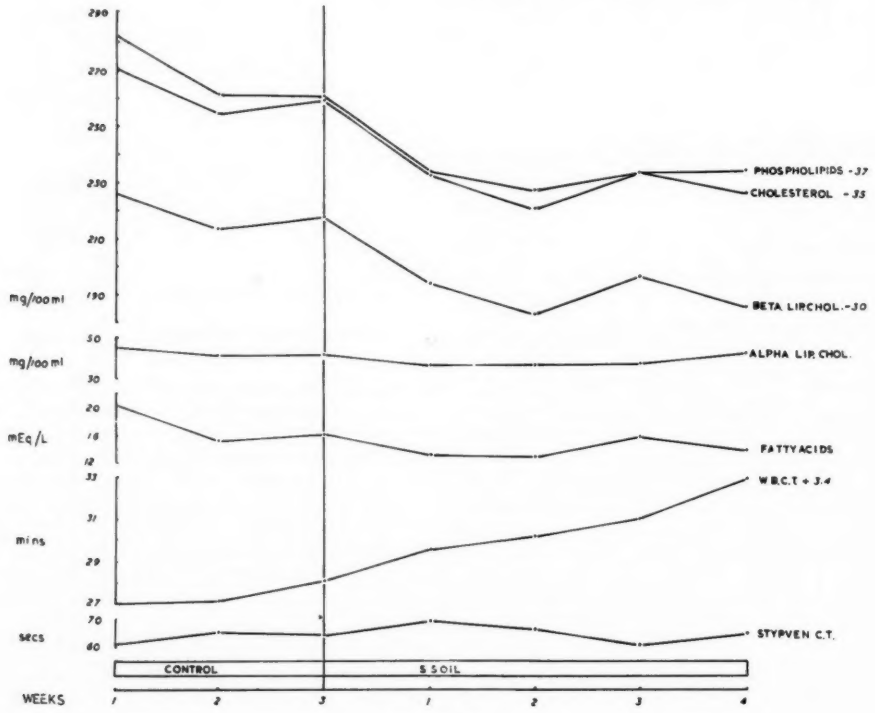

FIGURE II

The effects of sunflower-seed oil on the serum lipids and measures of blood clotting in atherosclerotic men

Comparisons of Blood Clotting between the Healthy Controls and the Atherosclerotic Subjects

The W.B.C.T. in both groups of subjects were almost identical when saturated fats were being consumed. During the administration of oil, the W.B.C.T. in the atherosclerotic subjects became significantly longer than in the control group.

Lack of response of the "Stypven" time to diet allowed data from both periods to be pooled. The "Stypven" time in the control group then averaged 69·2 seconds and was significantly longer ($P<0·05$) than the average of 64·2 seconds in the atherosclerotic subjects.

Because the various lipids measured—namely, cholesterol, phospholipids and fatty acids—exist in serum as constituents of lipoproteins in fairly constant proportions (Bragdon *et alii*, 1956), they cannot be regarded as truly independent variables. For this reason, calculations of multiple associations between the measures of blood clotting and the above-mentioned lipids cannot be performed, and the statistical methods are limited to simple correlations and to regression equations involving few variables.

Lipid-Clotting and Clotting-Clotting Relationships: Healthy Controls (Table II).—There is

TABLE II

Correlation Coefficients (with Levels of Significance) for the Serum Lipids and Clotting Times Measured in Healthy Young Men and Derived from Data Pooled from Both Dietary Periods; 63 Observations

Characteristic	Fatty Acid Concentration	Phospholipid Concentration	Alpha Cholesterol Concentration	Beta Cholesterol Concentration	W.B.C.T.	"Stypven" Clotting Time
Cholesterol concentration ..	+0·303*	+0·786***	+0·355**	+0·892***	−0·264*	+0·106⁻
Fatty acid concentration ..		+0·346**	−0·269*	+0·479***	+0·265*	−0·282*
Phospholipid concentration			+0·541***	+0·584***	−0·426***	+0·249⁻
Alpha cholesterol concentration				−0·101⁻	−0·508***	+0·461***
Beta cholesterol concentration					−0·030⁻	−0·117⁻
W.B.C.T.						−0·206⁻

* $P<0·05$; ** $P<0·01$; *** $P<0·001$; "⁻", not significant.

no significant relationship between the speed of clotting in the "Stypven" test and the W.B.C.T. This confirms earlier observations in a larger number of subjects (Goldrick and Whyte, 1959), and an explanation for this inconsistency can be found in the lipid-clotting associations. The correlation coefficient of alpha lipoprotein cholesterol concentration and "Stypven" time is positive and significant: that is, as alpha lipoprotein concentration increases the "Stypven" time becomes longer. On the other hand, the coefficient relating alpha lipoprotein cholesterol concentration to the W.B.C.T., while significant, is of opposite sign, suggesting that as alpha lipoprotein concentration increases the W.B.C.T. becomes shorter. Thus the "Stypven" time and the W.B.C.T. appear to react to alpha lipoprotein in opposite directions.

It will be noted that cholesterol, phospholipids and fatty acids are significantly related to the measures of blood clotting. Presumably, these relationships merely reflect the strong association of clotting with alpha lipoprotein, which carries part of the cholesterol phospholipid and fatty acid content of serum.

Regression equations relating the clotting times to the cholesterol content of alpha and beta lipoproteins (milligrammes per 100 ml.) were:

y "Stypven" (seconds) $=52·76+0·493$ alpha$-0·037$ beta

and

y W.B.C.T. (minutes) $=58·77-0·181$ alpha$-0·014$ beta

In both equations the regression coefficients for alpha lipoprotein are highly significant ($P<0·001$), while those for beta lipoprotein are insignificant.

Lipoid-Clotting and Clotting-Clotting Relationships: Atherosclerotics (Table III).—The lipid-clotting and clotting-clotting relationships are different in these subjects as compared with those in the healthy controls. In atherosclerotic subjects, the coefficient relating the "Stypven" time to the W.B.C.T. is positive and statistically significant; that is, the "Stypven" time varies in the same direction as the W.B.C.T. Again, this association can be explained in terms of the lipid-clotting relationships. The correlation coefficients between alpha lipoprotein cholesterol concentration and both the "Stypven" time and the W.B.C.T. are positive and significant, while those relating beta lipoprotein cholesterol concentration to the clotting times are both significant and negative. Thus, as alpha lipoprotein cholesterol concentration increases, the "Stypven" time and the W.B.C.T. become longer; while, on the other hand, increases in beta lipoprotein cholesterol concentration are associated with a shortening of each of the clotting times.

The negative and statistically significant coefficients relating cholesterol phospholipid and fatty acid concentrations to the clotting times probably reflect the beta lipoprotein-blood clotting relationships.

Regression equations relating blood clotting to the cholesterol content of alpha and beta lipoproteins (milligrammes per 100 ml.) were:

y "Stypven" (seconds) $=81·31+0·205$ alpha$-0·126$ beta
(for both coefficients, $P<0·001$)

and

y W.B.C.T. (minutes) $=33·9+0·057$ alpha$-0·034$ beta
(for both coefficients, $P<0·05$)

The slope and position of the equations expressing the "Stypven" time and the W.B.C.T. in terms of the lipoprotein fractions could not be compared between the two groups of subjects, owing to lack of homogeneity in the data.

TABLE III

Correlation Coefficients (with Levels of Significance) for the Serum Lipids and Clotting Times Measured in Atherosclerotic Men and Derived from Data Pooled from Both Dietary Periods; 70 Observations

Characteristic	Fatty Acid Concentration	Phospholipid Concentration	Alpha Cholesterol Concentration	Beta Cholesterol Concentration	W.B.C.T.	"Stypven" Clotting Time
Cholesterol concentration ..	+0·839***	+0·914***	−0·358**	+0·974***	−0·326**	−0·548***
Fatty acid concentration ..		+0·918***	−0·459***	+0·858***	−0·312**	−0·508***
Phospholipid concentration			−0·388***	+0·905***	−0·319**	−0·566***
Alpha cholesterol concentration				−0·559***	+0·289*	+0·474***
Beta cholesterol concentration					−0·366**	−0·602***
W.B.C.T.						+0·400***

* $P<0·05$; ** $P<0·01$; *** $P<0·001$; "–", not significant.

The correlation coefficients clearly demonstrate relationships between the serum lipid concentrations and the measures of blood clotting which are not obvious on inspection of the weekly averages in Figures I and II. Moreover, these associations in atherosclerotic subjects differ in certain respects from those in the healthy controls.

Relationships between the Serum Lipids.—Differences between the subjects are not limited to lipid-clotting associations, but are also apparent in the relationships between the lipoproteins and their constituent lipids.

Among the healthy subjects (Table II) there is no significant association between alpha and beta lipoproteins; however, the coefficients relating them to total cholesterol concentration suggest that an increase in total cholesterol concentration involves an increase in both alpha and beta fractions. Regression equations expressing total cholesterol concentration in terms of alpha and beta cholesterol (milligrammes per 100 ml.) were:

$$y \text{ Total Cholesterol} = 176·25 + 0·794 \text{ alpha} \quad (P \text{ alpha} < 0·01)$$

and

$$y \text{ Total Cholesterol} = 52·26 + 0·960 \text{ beta} \quad (P \text{ beta} < 0·001).$$

It is evident that the high concentration of beta cholesterol relative to alpha cholesterol results in beta lipoprotein being by far the more important lipoprotein fraction contributing to variations in total cholesterol concentration.

In atherosclerotic subjects (Table III), not only are the lipoprotein fractions significantly and negatively associated, but also the correlation coefficient relating total cholesterol concentration to alpha and beta lipoprotein cholesterol concentration indicates that, as total cholesterol concentration increases, beta cholesterol concentration increases, but alpha cholesterol concentration actually decreases.

Regression equations expressing total cholesterol concentration in terms of alpha and beta lipoprotein cholesterol concentration (milligrammes per 100 ml.) were:

$$y \text{ Total Cholesterol} = 292·67 - 1·270 \text{ alpha} \quad (P \text{ alpha} < 0·01)$$

and

$$y \text{ Total Cholesterol} = 68·07 + 0·862 \text{ beta} \quad (P \text{ beta} < 0·001).$$

Attempts to compare the slopes and positions of equations expressing total cholesterol concentration in terms of the lipoprotein fractions in the groups of subjects were unsuccessful, owing to lack of homogeneity in the data. Nevertheless, the equations relating total and alpha cholesterol are certainly quite dissimilar in the two groups.

The significant associations between the lipoproteins and their constituent lipids merely reflect the constant but different compositions of the lipoprotein fractions. The differences in the lipid-lipid associations between the atherosclerotic subjects and the healthy controls are due partly to the differences in lipoprotein behaviour already referred to, and possibly also to differences in lipoprotein composition.

DISCUSSION

Abnormalities in the serum lipids of groups of individuals manifesting complications of atherosclerosis, when compared with age-matched controls, are said to be an elevation of the total cholesterol level (Lawry *et alii*, 1957) and the cholesterol-phospholipid ratio (Oliver and Boyd, 1953), a reduction in alpha lipoprotein level and a rise in beta lipoprotein level (Barr *et alii*, 1951; Jenks *et alii*, 1956), and an elevation of the serum triglyceride level (Albrink and Man, 1959). By the selecting of two groups of subjects, one young and possessing relatively healthy arteries and the other older and presumably more atherosclerotic, the above-

mentioned differences have to some extent been exaggerated, attaining statistical significance in this small number of individuals. This is related to the rise in serum cholesterol level (Adlersberg *et alii*, 1956) and beta lipoprotein level (Russ *et alii*, 1951) with advancing age. A control group of young men was chosen, rather than age-matched controls, because it is impossible to be certain that older, apparently healthy controls are indeed free from this very prevalent disease. Moreover, if it is postulated that disorders of blood coagulation are associated with atherosclerosis and its complications, then such associations would be more readily apparent in an exaggerated comparison of this design. Differences between healthy and atherosclerotic subjects and between youth and middle age may be synonymous.

While a great deal of importance has been placed on the differences in serum lipid and lipoprotein levels between apparently healthy and presumably atherosclerotic individuals, little attention has been paid to the relationship between the lipoprotein fractions themselves, and the relationship they possess with total cholesterol level. In the healthy controls it was noted that the concentrations of alpha and beta lipoproteins were independent of each other, although both fractions, particularly beta lipoprotein, contributed to an increase in total cholesterol concentration. Among the atherosclerotic subjects, on the other hand, as total cholesterol concentration increased, that of alpha cholesterol actually decreased, while that of beta cholesterol increased. Moreover, the negative correlation coefficient between the alpha and beta fractions was significant, supporting this inverse relationship.

The reasons for these differences in cholesterol transport are not known, but they may have some bearing on the pathogenesis of atherosclerosis. Of the two lipoprotein fractions, the beta component is considered the more atherogenic by virtue of its large size and heavy lipid content (Page, 1954). For any given serum level of cholesterol, to judge by the present findings, an atherosclerotic subject would carry less cholesterol in alpha lipoprotein molecules and more in beta lipoprotein than a healthy person. If his serum cholesterol concentration was higher than that of his healthy counterpart (as is commonly the case), then these differences would be exaggerated. The atherosclerotic man, then, transports more of his cholesterol in a potentially damaging form. If this small group of atherosclerotic subjects is a truly representative sample of the atherosclerotic population at large, we have a possible explanation for the low alpha and high beta lipoprotein levels which are characteristic of this disease.

Earlier observations on a larger group of healthy Australians and New Guinea natives (de Wolfe and Whyte, 1958; Goldrick and Whyte, 1959) indicated that alpha lipoprotein cholesterol concentrations remained relatively constant in Australians, regardless of variations in total cholesterol level, while in the natives, alpha cholesterol concentration increased when the total cholesterol concentration increased. No dietary manipulations were employed in those studies. It is therefore of interest to find that the healthy Australian subjects in the present study behaved rather like the natives of New Guinea. This could conceivably be fortuitous, resulting from sampling errors involved in the selection of the small number of controls; but we gain confidence in the validity of the conclusion from the fact that the results from the present study are based on multiple observations derived from these subjects during enforced variations in total cholesterol level. In neither the present nor the previous studies of healthy Australians has there been any demonstrable relationship between alpha and beta lipoproteins. It would seem reasonable to picture three stages in the lipid deterioration towards atherosclerosis. At the first stage there is the native who has little predisposition to atherosclerosis, whose serum cholesterol level is low, and in whom increments in serum cholesterol are carried by both lipoprotein fractions so that a constant cholesterol distribution is maintained. Secondly, there is the healthy Australian with his greater predisposition to atherosclerosis, whose serum cholesterol level is higher, and who carries nearly all increments in cholesterol in the beta fraction while the alpha fraction's content of cholesterol is almost constant. Then there is the atherosclerotic subject, whose cholesterol level is generally, but not always, higher still, and in whom an increase in cholesterol concentration is borne by the beta fraction while the alpha lipoprotein cholesterol concentration actually decreases.

It is of considerable interest that it was possible to demonstrate definite relationships between the serum lipids and blood clotting when measured under basal conditions with varying levels of blood fats. Observations in a larger group of Australians and in New Guinea natives (Goldrick and Whyte, 1959) suggested that the presence of alpha lipoprotein was associated with a lengthening of the "Stypven" time. Moreover, as an association between the W.B.C.T. and alpha lipoprotein was not then observed, the two clotting tests gave results

which appeared to be unrelated, and this lack of agreement seemed rather anomalous at the time.

The failure of either test of blood clotting to be affected by alteration in the serum lipid levels in the healthy controls appears to be due to the relative constancy of alpha lipoprotein concentration and to the lack of association between beta lipoprotein and clotting.

The longer W.B.C.T. in the atherosclerotic subjects when sunflower-seed oil was consumed are probably accounted for by the fall in beta lipoprotein level. The lack of change in the "Stypven" time is not readily explained on this basis, although it is of interest to note that other observers have failed to demonstrate any changes in this time after lowering of the serum cholesterol level in atherosclerotic subjects (McDonald and Edgill, 1958).

The shorter "Stypven" times in atherosclerotic subjects are in conformity with McDonald and Edgill's (1957) findings when atherosclerotics were compared with age-matched controls. Possibly this is accounted for by the lower alpha lipoprotein levels found in this disease.

More than one interpretation of these associations between the lipoproteins and the measures of blood coagulation is possible. Either the lipoproteins themselves are able to influence clotting, or, though they are inactive themselves in this regard, their concentrations vary in parallel with other, perhaps chemically unrelated, factors which do possess coagulant and anti-coagulant properties. If the second interpretation is correct, then the hypothetical factor(s) related to alpha lipoprotein must be widely distributed, since it is present in natives of New Guinea and in healthy and atherosclerotic Australians.

There is no direct evidence to suggest that the lipoproteins or fasting blood possess coagulant or anticoagulant properties; but there is ample evidence to show that certain lipids are able to influence the rate of blood clotting. Cholesterol itself, triglycerides, amino acids and the majority of phospholipids have no influence on clotting systems *in vitro*, but long-chain unesterified fatty acids and phosphatidyl ethanolamine (P.E.) accelerate clotting (Rouser *et alii*, 1958). P.E. or P.E.-like compounds are probably responsible for the coagulant properties of chylomicra *in vitro* (Poole, 1955; Poole and Robinson, 1956; Robinson and Poole, 1956). Lysophosphatides are inhibitors in the "Stypven" test (Maclagan *et alii*, 1958) and are constituents of lipoproteins present in highest concentration in the high density (alpha) fraction (Phillips, 1959). However, the specific lipid constituents of the complex lipoprotein molecules in health and disease are not sufficiently well characterized to enable one to predict whether lipoproteins can be classified as clotting factors. Perhaps studies currently being undertaken *in vitro* with the use of purified lipoproteins will settle the issue, and provide an explanation for the different lipid-clotting associations which have here been found in healthy and atherosclerotic subjects.

Although there are uncertainties in the interpretation of the lipid-clotting associations, the observations are of interest in regard to atherogenesis. Lipoproteins are sometimes regarded as important factors in the development of this disease, and the serum concentrations of the various fractions are presumed to provide an indication of the tendency for lipid to be deposited in the intima (Page, 1954). Even if it is denied that lipoproteins influence blood coagulation, at least they do change and can be made to change along with other factors which do influence coagulability. Therefore they can be regarded as measures of the tendency to form fibrin. Hence, the concentrations of the various lipoprotein fractions reflect not only the tendency for vascular damage to occur by lipid infiltration, but also the likelihood of intimal changes occurring from the deposition of fibrin (Duguid, 1946, 1948).

If this small group of atherosclerotic subjects is a representative sample, and provided that the inhibition of clotting persists for as long as the reduction of the serum lipid concentration is maintained, then, on theoretical grounds at least, lowering of the serum lipid concentration in this disease should be of value in preventing thrombotic complications.

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THE "Q" WAVE AND HIGH CHEST LEADS: AN ANALYSIS OF 425 CASES[1]

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SUMMARY

Normal *Q* waves indistinguishable from abnormal *Q* waves may be recorded from leads on the chest wall. They differ only in site, but not in form or duration.

The mechanism of production of *Q* waves is discussed, and the findings at two fixed points on the upper part of the anterior chest wall are analysed in 425 cases.

It is clear that the normal *Q* zone rarely extends to the second intercostal space above the V_2 position, or the middle of the sternum at the level of the third intercostal space. Only two instances of *Q* waves were seen at this level in 149 normal subjects.

In none of 32 cases of posterior infarction were *Q* waves found in these leads, whereas in 85% of cases of recent anterior infarction *Q* waves are found at these sites.

The presence of a *Q* wave in these leads is highly unlikely to be normal if bundle branch block, ventricular hypertrophy, gross clock-wise rotation and right ventricular dilatation are excluded.

THE purpose of the present investigation is to determine the value of high anterior chest leads in clinical electrocardiography. It has sometimes been suggested that leads be taken from other than the standard chest positions to seek out *Q* waves when there is an equivocal electrocardiographic pattern in a suspected case of myocardial infarction.

This procedure in general is to be deprecated, as normal *Q* waves of 0·04 second duration exist on the chest wall and are indistinguishable in appearance from abnormal *Q* waves. The key to the problem is an understanding of the mechanism of production of a *Q* wave, and of the reason why its presence in certain areas of the chest is abnormal and denotes infarction.

This study also deals with the patterns found in leads situated at two fixed points on the upper part of the anterior chest wall.

When transmural infarction takes place in the left ventricular wall, early instantaneous *QRS* vectors are lost from the infarcted area and fail to contribute to the *QRS* loop (Wilson *et alii*, 1931). This produces a deviation of the early portions of the loop up to the 0·04 second time interval in a direction away from the infarcted area. In a single infarction this would correlate with the anatomical site of the lesion. However, as infarction is frequently multiple and the early *QRS* vectors are the resultant of the effects of all the infarcted areas, such anatomical correlation is by no means constant.

On the assumption that the heart, electrically, can be treated as an equivalent dipole (Frank *et alii*, 1955), the axis of the dipole is a flow line and perpendicular to it, and midway between the poles is an iso-potential line of theoretically zero potential which, if extended, will cut the body surface and divide the body into electro-negative and electro-positive zones (Figure I).

Thus all V leads on the chest surface, no matter where situated, record either positive, negative or zero (isoelectric) potential for any given instant in the cardiac cycle. By multiple surface recording, a number of points are found which give a zero potential at any one instant in the *QRS* cycle. These points must all lie on a plane which is at right angles to the cardiac vector at that moment. This is called the null plane. Leads on either side of this plane must record either positive or negative deflections. Whilst the null or dividing plane usually inscribes a regular ellipse around the chest, a significant proportion of deviations from this does occur, possibly owing to eccentricity of the dipole in the chest, the lack of true homogeneity of the conducting medium, and variations in chest shape, so that the null plane concept, as a basis for electrocardiography, is only approximately valid (Langer *et alii*, 1953). However, if the *QRS* complex is subdivided into early and late vectors corresponding to the *Q* wave and

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S wave, the method has value (Grant, 1957), particularly since routine precordial exploration is sufficient (except in problem cases) to determine the path of the null plane (Grant, 1950, 1957).

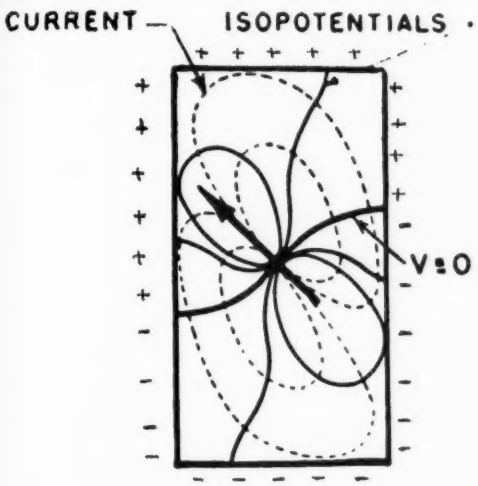

FIGURE I

Current lines (interrupted) and iso-potentials are shown in cross-section for an homogenous conducting cylinder in which is immersed a centric current dipole. (*After* Kossman, 1958)

As the instantaneous vectors of the *QRS* can be analysed at 0·01 second intervals, it is found that the null plane of the body moves through a considerable angle, and only a small portion of the chest wall has no electro-positivity during the first 0·04 second. This is called

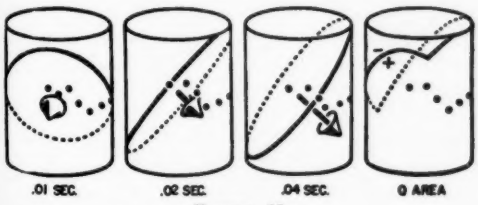

FIGURE II

Normal *Q* area, null plane pathways plotted for three instants during first 0·04 second with *Q* area on the right. The position and size of this area vary considerably in normal subjects. (Grant 1958)

the *Q* zone of the chest; it differs only in stei from the infarction *Q* zone, and normally lies over the right anterior aspect of the chest and the upper back area on the right side (Grant and Murray, 1954; Wolf *et alii*, 1954; Figures II, III and IV). In this area *Q* waves of 0·04 second can be recorded. Grant and Murray describe five sites in which abnormal *Q* waves are found. They are: (i) strictly anterior; (ii) antero-lateral; (iii) inferior or diaphragmatic; (iv) strictly posterior; (v) high lateral. If infarction *Q* waves lie outside the reach of the conventional twelve-lead electrocardiogram, a definite diagnosis of infarction cannot be made

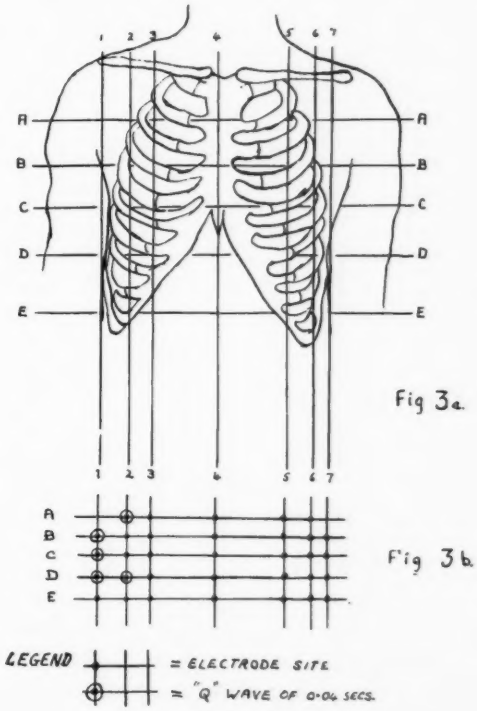

FIGURE III

(*a*) Exploration of the anterior chest wall for *Q* waves of 0·04 second duration in normal subjects. Electrode sites 1 and 7 mid-axillary, 2 and 6 in the anterior axillary line, 3 and 5 in the mid-clavicular line, 4 in the mid-sternal line. Horizontal lines at intervals of 5 cm., commencing at the second intercostal space. (*b*) Sites where *Q* waves of 0·04 second interval were recorded. *Q* zone to the right of the anterior axillary line. Normal male subject, aged 29 years. See Figure IV

from the tracing, although non-specific changes may result in a diagnosis of myocardial ischæmia, coronary insufficiency or non-specific changes. The five zones mentioned are by no means fixed, owing to the marked variations in arborizations of the coronary arteries (Schlesinger, 1940) and to the altered relationships of arterial branches to the segments of the myocardium which occur in persons who have had arteriosclerosis.

Surveys of the chest using 96 to 117 electrode sites have been undertaken by Prinzmetal *et alii* (1957), and a 40-lead record has been analysed by Grant and Murray (1954) as a research measure to delineate the extent of *Q* waves on the chest.

using special electrode placements on the chest (Nehb *et alii*, quoted by Kossman, 1958; Trethewie, 1953, 1959).

On the other hand, others such as Wilson *et alii* (1944) and Myers (1956) have recognized the necessity for leads in addition to the conventional

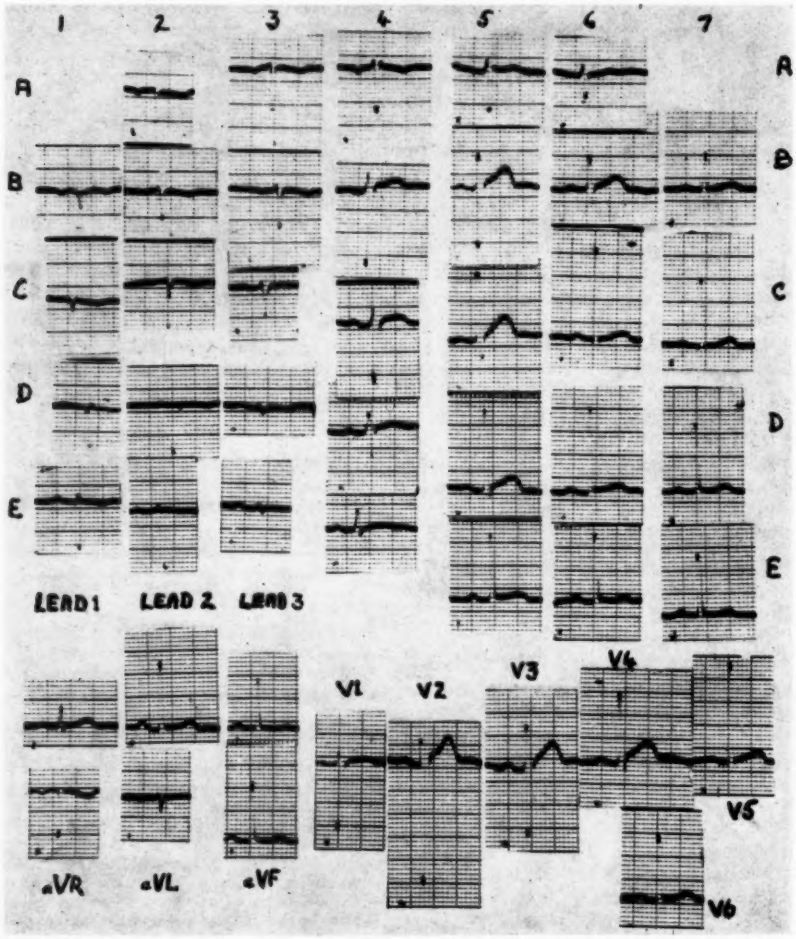

FIGURE IV

Normal male subject, aged 29 years. Exploration of the chest at 33 sites. *Q* waves visible on the right anterior aspect of the chest, and also in the right arm lead. Leads taken from points marked as in Figure III (*b*)

The importance of attempting to delineate the *Q* areas of high anterior infarction lies in the frequent correlation of a good prognosis with this type of infarction (Prinzmetal *et alii*, 1957).

Many investigators have tried to better the diagnostic value of the conventional leads by unipolar precordial leads, and have taken tracings at intersections of horizontal lines drawn from the level of the sternal end of the second, third and fourth intercostal spaces, with the vertical projections drawn through the usual precordial sites. Others, such as Odle

et alii (1950) have used supraclavicular sites, and quite a number (Burchell, 1948; Oram *et alii*, 1951) have used the œsophagus, while Lambert (1953) has explored the abdomen and taken unipolar leads from the ensiform process and from the umbilicus.

Prinzmetal *et alii* (1957) point out that if the *rS* patterns found in the second intercostal space above V_2 and at a mid-sternal site at the level of the third intercostal space are replaced by *Q* waves, then a diagnosis of high anterior infarction can be made. Massumi (1957) has reviewed 106 normal subjects and found only

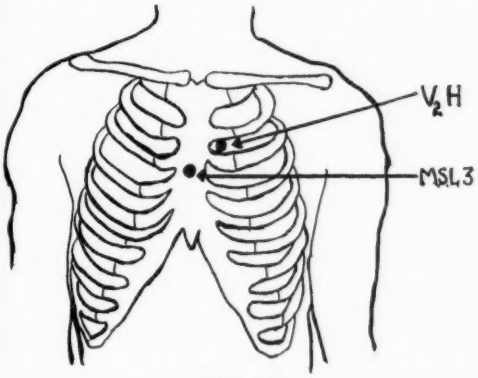

FIGURE V

High chest leads V_2H at second intercostal space above the V_2 position. MSL_3, mid-sternal line at the level of the third intercostal space

one with evidence of a *Q* wave in the second intercostal space above *V*1, and none at all with a *Q* wave in the mid-sternal line at the level of the third intercostal space. This applies only when gross clockwise rotation, right ventricular hypertrophy or dilatation, right and left bundle branch block or left ventricular hypertrophy have been excluded.

PRESENT INVESTIGATION

Twelve-lead electrocardiograms were taken from 425 patients, and in addition leads were taken from the second intercostal space directly above the V_2 position, and a further tracing was taken from the mid-sternal line at the level of the third intercostal space. These respectively will be designated V_2H and MSL_3 (see Figure IV). The 425 subjects were grouped as follows:

Normal	149
Myocardial infarction	82
Left ventricular hypertrophy	62
Myocardial ischæmia	73
Right bundle branch block	8
Left bundle branch block	16
Miscellaneous	35

This classification was based on the findings of the routine twelve-lead electrocardiogram using current standards (Friedberg, 1956). An analysis of the results appears in Tables I, II and III. With regard to the *Q* waves, it is apparent that in no case of posterior infarction, either recent or old, were *Q* waves found in the V_2H or MSL_3 position. On the other hand,

TABLE I

Findings in 149 Normal Subjects

Wave	V_2H[1]	MSL_3[1]
Q	9	2
R	32	37
r	108	110
Inverted *T*	32	26
S-T elevation greater than 1 mm.	16	23

[1] For explanation, see text.

TABLE II

The Incidence of Q Waves in High Chest Leads in 82 Cases of Infarction

Type of Infarction	Total Number of Subjects	*Q* Waves Present	
		V_2H	MSL_3
Old:			
Anterior	23	11	12
Posterior	15	0	0
Recent:			
Anterior	27	23	23
Posterior	17	0	0

TABLE III

The Q Wave in High Chest Leads in Various Abnormalities

Abnormality	Total Number of Subjects	*Q* Wave Present	
		V_2H	MSL_3
Myocardial ischæmia	73	8	5
Left ventricular hypertrophy	62	10	6
Left bundle branch block	16	8	8
Right bundle branch block	8	1	1
Miscellaneous	35	2	2

in 23 out of 27 cases of recent anterior infarction *Q* waves were present, and in slightly over half of the cases of old anterior infarction a *Q* wave was present in both the V_2H and MSL_3 positions. With regard to the normal subjects only nine out of 149 had a *Q* wave in the V_2H position and two in the MSL_3 position. Of the 73 subjects of myocardial ischæmia, eight showed a *Q* wave in the V_2H position and five in the MSL_3 position. Of the 62 subjects with left ventricular hypertrophy, 10 had a *Q* wave in the V_2H position and six in the MSL_3 position.

Of the 16 patients with bundle branch block on the left side, eight had *Q* waves in these two leads, whereas of the nine with right bundle branch block only one had a *Q* wave. In the miscellaneous group of 35 cases consisting mainly of atrial fibrillation and atrial hypertrophy, there were only two subjects with *Q* waves in the high chest leads.

Discussion

Of the two subjects among the normal group who showed *Q* waves, one was found to have suffered from diffuse arteriosclerosis and was aged 80 years. Details of the other normal patient with *Q* waves were not obtainable.

The method of selection of normal subjects has been based in this paper on the twelve-lead standard electrocardiogram and not on clinical grounds. Prinzmetal *et alii* (1957) have shown that in old antero-septal and anterior infarcts, high sternal leads may disclose a residual *Q* area, derived from the larger *Q* area present at the time of the acute infarction. Massumi (1957), on the other hand, using persons with normal hearts, found no example of a *Q* wave in the third intercostal space from the V_1 to the V_4 positions, and only one in the second intercostal space from the V_2 to V_4 positions.

Hence it is possible that these two normal subjects, and some of the ischæmic group who showed *Q* waves, may well have had old anterior infarcts. The reason for this assertion lies in the fact that it has been frequently shown that in normal subjects the initial portion of the *QRS* loop is expected to point superiorly, anteriorly and to the right and give initial positivity in the *QRS* complexes recorded in the high sternal area (Grant and Murray, 1954; Wolff *et alii*, 1954).

Thus it would appear that *Q* waves in the high chest leads in normal people are distinctly rare, the incidence being just over 1%.

Analysis of the *S-T* interval showed that an elevation of more than 1 mm. was not uncommon among the normal subjects occurring in 16 cases in the V_2H position and in 23 cases in the MSL_3 position. It is of interest that in the cases of right bundle branch block there was no elevation of the *S-T* interval, whereas in the cases of left bundle branch block elevation occurred in each case, and often markedly so.

The *T* waves were quite variable, being inverted in 32 cases in the V_2H position and in 26 in the MSL_3 position among the normal subjects, and among the patients with left ventricular hypertrophy there were respectively eight and five with *T* wave inversions in the V_2H and the MSL_3 positions, but only once was inversion related to a *Q* wave. Among the infarction group, *T* wave inversion was not consistent; it occurred in 15 cases among the 82 in this group. Anterior infarction, both recent and old, seemed to produce more cases of *T* wave inversion than recent posterior or old posterior infarction. Among the group of patients with myocardial ischæmia, *T* wave inversion was present 15 and 17 times respectively in the V_2H and MSL_3 positions.

Summary

Whilst the *S-T* and *T* waves in the high anterior chest positions appear to be of no diagnostic significance, it is apparent that the *Q* waves are fairly highly significant, in that it is extremely rare for normal people to have a *Q* wave in either the V_2H or MSL_3 position.

On the other hand, it is quite common for patients with anterior infarction to have such a *Q* wave, and according to Prinzmetal *et alii* (1957) this *Q* wave was the only evidence of high anterior infarction in six cases, whilst the conventional leads in 66 cases were non-specific. This present analysis was not conducted with a view to diagnosing myocardial infarction by the high chest leads in cases in which the infarct was not obvious from the precordial or standard leads, and it is possible that among the group of patients listed as suffering from myocardial ischæmia a certain percentage really had infarction, as revealed by *Q* waves in the upper chest leads. However, this is conjecture, and the problem would need to be tackled by a prospective form of analysis. From this study it would appear that exploration at the V_2H and MSL_3 positions is indicated when there is conflict between the electrocardiographic findings and the clinical findings in a suspected case of myocardial infarction. The finding of a *Q* wave in these leads is uncommon in normal subjects, and would increase the suspicion of infarction, provided bundle branch block, ventricular hypertrophy, right ventricular dilatation and gross clock-wise rotation of the heart were excluded.

Lastly, the importance of vectorial analysis based on the dipolar concept is emphasized, to help in the understanding of *Q* waves in the standard electrocardiogram.

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CHRONIC PYELONEPHRITIS AND HYPERTENSION[1]

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SUMMARY

The autopsy incidence of chronic pyelonephritis has been investigated in a series of 200 hypertensive patients. Evidence of the disease was found in 51·5%, the condition being unilateral in 4·5%. The findings indicate that "essential" hypertension is rare in young patients. Malignant hypertension and uræmia should suggest the presence of primary renal disease. Only 10% of the cases were diagnosed clinically, and the condition is often overlooked or misdiagnosed pathologically.

The suggestion is put forward that full urine examination, X-ray investigation of the abdomen and intravenous pyelography should be used more often and with greater care, in order that the clinical recognition of the disease may be improved.

"ESSENTIAL" HYPERTENSION is a very common clinical diagnosis. Efforts on the part of clinicians to exclude causes of hypertension give negative results in the majority of instances. Autopsy examination of hypertensive patients uncovers primary renal disease frequently in cases in which clinical investigations have failed to do so. This applies in particular to patients in younger age groups. The commonest unsuspected renal disease is chronic pyelonephritis.

The present study was undertaken in order to discover the discrepancy between clinical and pathological "essential" hypertension. This followed an impression that the discrepancy was very great.

An incidental observation has been that many pathologists do not recognize chronic pyelonephritis when evidence of it is clear. The criteria of diagnosis must be reemphasized because of the important place the disease occupies in the broad subject of hypertension. There is also evidence that a greater awareness of the condition is needed on the part of clinicians. This may lead to more frequent discovery of the disease by more careful investigation of the renal tract in hypertensive patients. Treatment of the latent disease in hypertensives may be of value.

MATERIAL

During routine autopsies in the Dunedin Hospital from 1953 to 1956, unsuspected chronic pyelonephritis was often found, particularly in hypertensive subjects. This study was undertaken to discover the incidence in a consecutive series and to analyse the results in relation to the clinical findings.

The renal lesions were studied in a series of 200 consecutive autopsies on hypertensive subjects. Some of the examinations were conducted personally, and in a considerable number the entire kidneys were available. In all the cases, paraffin blocks from both kidneys were studied. Hæmatoxylin and eosin and Van Gieson stains were used as a routine, while Van Gieson elastic and P.A.S. stains were added when indicated to examine special features.

Clinical details and gross pathological information were extracted from the autopsy records in the Otago Medical School Pathology Department.

CRITERIA OF HYPERTENSION

If the blood pressure had been repeatedly recorded as 180 mm. Hg systolic or over, this was regarded as sufficient evidence for a case to be included in the series, irrespective of the condition of the heart at autopsy. If clinical information was not clear, the conditions laid down by Fishberg (1954) were applied. Heart weights of 400 grammes in females and 450 grammes in males were taken to indicate hypertension if other causes of the enlargement were excluded. When the wall of the left ventricle measured 1·5 cm. or more in basal thickness, this was regarded as confirmatory evidence.

CRITERIA OF CHRONIC PYELONEPHRITIS

The classical papers of Staemmler and Dopheide (1930) and of Weiss and Parker (1939)

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firmly established the histological criteria whereby chronic pyelonephritis may be differentiated from other causes of renal scarring. Confirmation from animal experiments was soon forthcoming from the studies of Mallory *et alii* (1940). More recently, the contributions of De Navasquez (1950, 1956, 1958) and Kincaid-Smith (1955) have reaffirmed the validity of the pathological criteria.

Nevertheless, it is apparent that many authors do not attach significance to this evidence. For example, in the papers of Canny (1940), Saphir and Ballinger (1940), Kahn and Laipply (1942), Higbee (1944), Perry (1945), Shea *et alii* (1948), Heptinstall (1953), Howard *et alii* (1954), and Hunter and McElmoyle (1956) the diagnosis appears to have been overlooked or mistaken for other causes of renal scarring. In particular, the renal lesions have too often been attributed to ischæmia. From limited observations in New Zealand, the vague diagnosis of "nephrosclerosis" is made not infrequently in cases in which the classical features of chronic pyelonephritis are evident. This has tended to prevent the condition from receiving the attention it deserves from clinicians.

Macroscopic Features

The coarsely scarred and nodular kidney with thickened pelvis does not give rise to difficulty in diagnosis. When the pelvis has been spared, the ætiology of coarse scarring becomes controversial (Figure I). Differentiation from healed infarcts and congenital grooves is usually possible on histological examination. Marshall (1953, 1956) has drawn attention to a theory that many renal cortical scars represent foci of congenital renal agenesis. Interpretation of the appearances is difficult, particularly when infection is superimposed. The present study supports the views of De Navasquez (1958) and of Bell (1946), that most renal scars are infective in origin.

In this series, histological evidence of chronic pyelonephritis has been found in kidneys showing a wide variety of gross appearances. Of the kidneys from cases of chronic pyelonephritis, 53·8% were described as showing coarse lesions of irregular distribution. In the remainder, a regular diffuse granularity of the surfaces was noted. This was described as fine in 40·6% and coarse in 5·6%. Diffuse, finely distributed lesions were described by Weiss and Parker (1939), by Mallory (1940) and by Bell (1946). It is stressed that the condition may not be suspected on macroscopic examination of the kidneys in the cases which resemble benign nephrosclerosis (Figure II).

In the group in which coarse irregular scars were present, the changes indicating chronic infection were often seen in the scarred areas only on histological examination. The diagnosis may be overlooked if only the renal tissue between the scars is examined. Platt and Davson (1950) drew attention to this important fact.

From the study, it is emphasized that the recognition of chronic renal infection may often depend on careful histological study, and that gross appearances may be misleading.

Microscopic Features

It was noted that the classical microscopic features of the disease were in the majority of cases irregularly distributed, and that foci of inflammation and scarring had no relation to the distribution of vessels. In many cases, vascular lesions were present in addition, but these did not prevent recognition of the underlying condition. The criteria of diagnosis to which particular significance was attached were as follows.

Periglomerular Fibrosis. — Concentric pericapsular fibrosis associated with survival of glomerular capillary tufts was regarded as typical of chronic inflammation (Figure III). In acute pyelonephritis, the inflammatory exudate is often seen around intact glomeruli. When such exudates become organized, the picture of periglomerular fibrosis results.

Atrophic Tubules Containing "Colloid" Casts.—When groups of tubules distended with dense eosinophilic casts were seen, this was regarded as indicating past infection (Figure IV). According to Mallory (1940) and Kincaid-Smith (1955), the casts are derived from polymorphonuclear leucocytes (Figure V). Nuclear remnants were frequently observed in the casts during this study (Figure VI), and no other explanation for the classical appearance (*struma renalis*) has been forthcoming. It would seem reasonable that the final picture results after an interval during which the affected tubules have become obstructed by scar tissue.

Interstitial Inflammation.—Foci of lymphocytes are commonly found in scarred kidneys and do not signify infection. However, if the inflammatory aggregations are haphazardly distributed and vary in extent and depth, chronic infection should be suspected (Figures VII and VIII). If the exudate includes plasma cells and/or eosinophils, this provides certain evidence.

Pelvic Inflammation.—If the tissues beneath the pelvic epithelium are scarred or chronically inflamed, the diagnosis of chronic pyelonephritis

FIGURE I
Bilateral chronic pyelonephritis. Coarse scars and nodules are shown

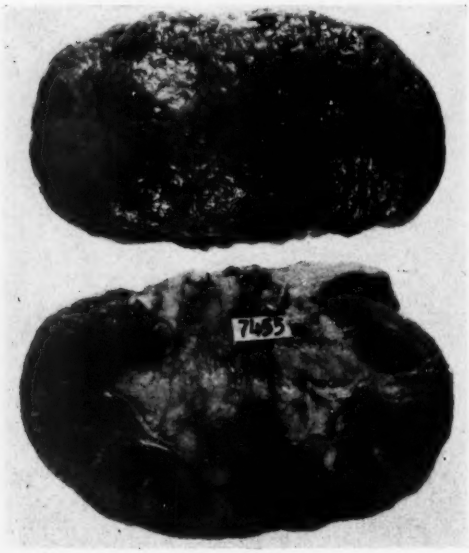

FIGURE II
Bilateral chronic pyelonephritis. Lesions diffusely distributed. Such an appearance resembles that of benign nephrosclerosis or chronic glomerulonephritis

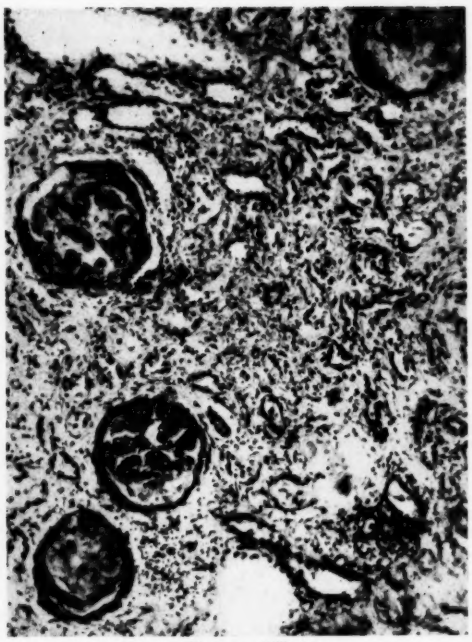

FIGURE III
Concentric periglomerular fibrosis. Capillary tufts survive. (Van Gieson, ×75)

FIGURE IV
Atrophic tubules containing dense "colloid" casts (*struma renalis*). (Van Gieson, ×90)

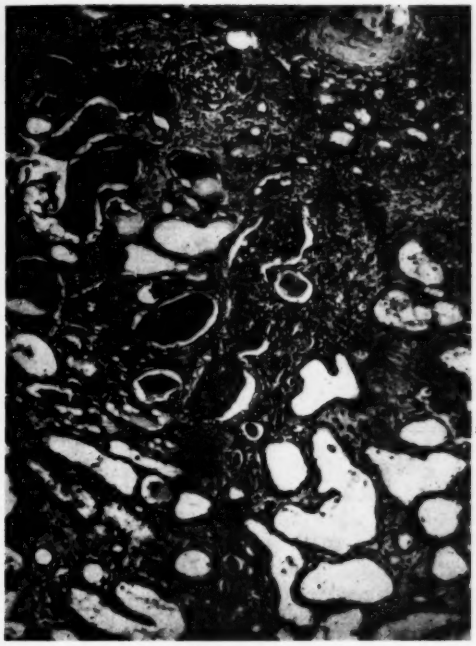

FIGURE V

Cellular cast in the centre of the field, adjacent to dense "colloid" casts. (Van Gieson, ×135)

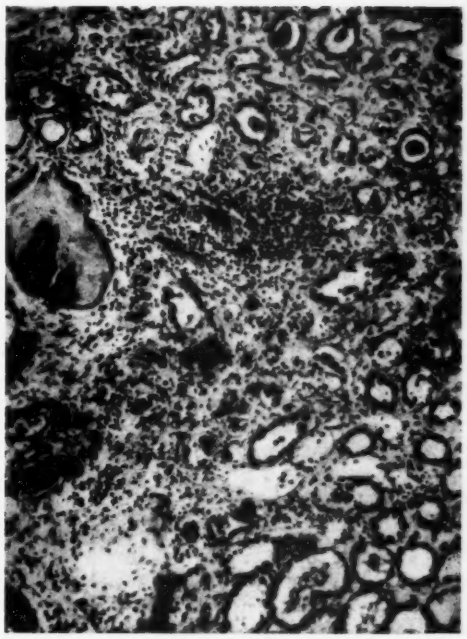

FIGURE VII

Interstitial inflammation and scarring adjacent to atrophic tubules. (Hæmatoxylin and eosin, ×90)

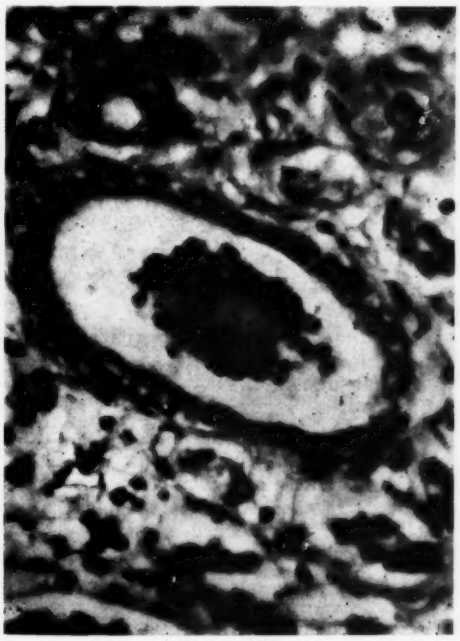

FIGURE VI

Dense cast surrounded by nuclear debris. This appearance is commonly seen in long-standing chronic pyelonephritis. (Hæmatoxylin and eosin, ×500)

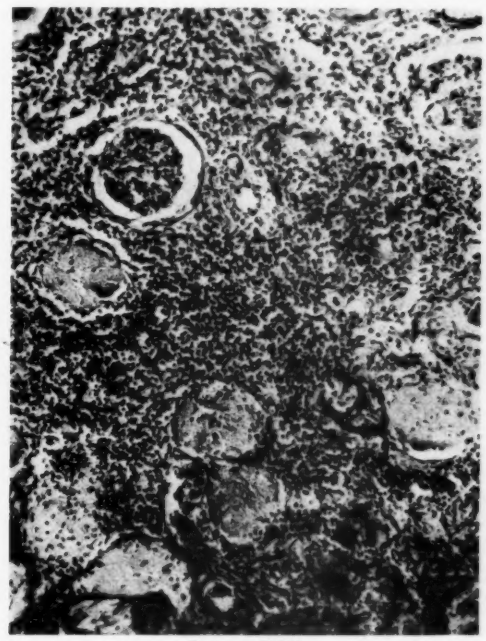

FIGURE VIII

Intense interstitial inflammation, surrounding obliterated glomeruli. (Van Gieson, ×90)

TABLE I

Age (Years)	Benign Nephrosclerosis		Chronic Bilateral Pyelonephritis		Unilateral Pyelonephritis		Other Lesions	
	Male	Female	Male	Female	Male	Female	Male	Female
31–40 ..	—	1	2	2	1	2	1	1
41–50 ..	9	2	3	4	—	—	1	—
51–60 ..	6	10	4	8	1	—	2	1
61–70 ..	8	13	9	11	2	—	1	1
71–80 ..	18	14	20	16	—	3	1	1
81–90 ..	4	5	8	6	—	—	—	—
91–100 ..	2	—	1	—	—	—	—	—
Total	47	45	47	47	4	5	6	4
	46%		47%		4·5%		5%	

is confirmed. However, in cases of hæmatogenous origin the pelvis may be spared. This criterion was regarded as not essential for the making of a diagnosis. The term pyelonephritis has been used loosely in this connexion throughout the study, because of commonly accepted practice. It is suggested that some title such as interstitial nephritis or focal nephritis should be employed for the apparently hæmatogenous condition.

RESULTS

The results of the study are summarized in Table I. The cases are analysed according to the findings in the kidneys and are grouped according to the subject's age and sex.

DISCUSSION

Age Incidence of Lesions

It is noted that only one case of benign nephrosclerosis (essential hypertension) was found in a patient aged under 40 years. In this younger age group there were eight cases of chronic pyelonephritis and one of chronic glomerulonephritis. Platt (1948), Platt and Davson (1950) and McMichael (1952) have stated that hypertension in young persons is nearly always secondary to some renal disease.

There is also clear evidence that chronic renal infection is not infrequent in older patients, in whom "essential" hypertension is more likely to be diagnosed.

Incidence of Chronic Pyelonephritis

The disease was found in 103 cases out of the total, an incidence of 51·5%. This is as high as any previously recorded (Crabtree, 1940). In 94 of these the disease was bilateral. From a study of clinical records in these cases, the condition was suspected or diagnosed in only 10 instances (10%). It is apparent that the disease is often present without manifesting itself clinically. Thirty-four years ago Fishberg (1925) made an observation of a similar nature, when he wrote:

> We are occasionally confronted at necropsy by a secondary contracted kidney, despite the fact that the clinical features of the case gave no indication that the hypertension was associated with inflammatory disease of the kidneys—the definition of essential hypertension is defective as it defines the disease solely by exclusion.

Braasch (1938) stressed that the condition might often be present without clinical manifestations, and that even urographic examination might give negative results. Attention has been directed to the renal tract in hypertensive patients by Schroeder and Steele (1938), by Mulholland (1939), by Wosika and Maher (1939), by Bothe (1939) and by Crabtree and Prein (1939). Since then, many authors have expressed the view that pyelonephritis is an important condition relating to hypertension. The papers of Weiss and Parker (1939), McCann (1940), Crabtree (1940), Shure (1942), Wosika *et alii* (1942), Likely *et alii* (1942), Boyd (1942), Smith *et alii* (1943), Hayes and Ashley (1943), Smith (1948), McManus (1950), Platt and Davson (1950), Stansfeld (1954) and Kipnis *et alii* (1955) are examples.

Recent work by Kincaid-Smith (1955), Kass (1955), Sophian (1956), Brod (1956), Derow (1956) and Brainerd and Cecil (1956) has served to draw again the attention of clinicians and pathologists to the latent and insidious aspects of pyelonephritis.

Ætiology of Hypertension

The mechanism whereby hypertension develops in some cases of chronic renal infection

has not become apparent during the study. Lesions of small vessels—namely, arteriolosclerosis and elastic hyperplasia—were present in the majority of cases, while arteriolonecrosis and cellular hyperplasia were seen in a few. These were regarded as resulting from the hypertension, as they bore no relation to the lesions ascribed to chronic infection.

Narrowing, scarring and obliteration of large branches of the renal artery were extremely variable, and bore no constant relation to the severity or duration of the clinical blood pressure. The degree of renal contraction and interstitial scarring were also inconstant in this regard. Ischæmia, therefore, does not appear to be the only factor in the initiation of the hypertension. Rosenheim (1954) cited a case of severe hypertension, in which only a small focus of infection involved one pole of one kidney. The hypertension was relieved by nephrectomy. On the other hand, severely contracted pyelonephritic kidneys occur in the absence of hypertension. Kincaid-Smith (1955), however, found that there was an association between the severity of vessel lesions and hypertension in a series of cases of pyelonephritis. She produced evidence of severe damage to large vessels during the acute phase of the infection, which resulted in narrowing and occlusion as healing progressed. A relationship between severe pyelonephritis, scarring and hypertension was established experimentally in rabbits by Heptinstall and Gorrill (1955).

A causative relationship between hypertension and past infection cannot be established in many cases in this series. A strong suspicion exists in the younger age group; but this becomes less apparent over the age of 45 years, when the incidence of "essential" hypertension becomes high.

Recognition of the condition in a hypertensive is still regarded as very important, as treatment of the condition is always warranted, whether surgical or with antibiotics. More cases might be discovered by more strenuous investigation of the kidneys in hypertensive patients by study of the cytological findings in the urine and by radiography.

Uræmia and Malignant Hypertension

No case of primary malignant hypertension was found in the series. When the characteristic vessel lesions were seen, they occurred as complications in association with pyelonephritis.

When renal failure had been present, glomerulonephritis or pyelonephritis was found in every case.

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An Essay Written by Request

THE ROLE OF PHYSICAL THOUGHT IN MEDICAL RESEARCH

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LAST year the *Reviews of Modern Physics* devoted two quarterly issues to reporting on current applications of physics to biology; it is almost two decades since Loofbourow in the same journal reviewed borderline problems between these sciences, and over two decades since Rashevsky's school in Chicago started the *Bulletin of Mathematical Biophysics*. In the same period we have seen the establishment of *Biochemica et biophysica acta*, *The Institution of Radio Engineers' Colloquia on Medical Electronics*, *Physics in Medicine and Biology*, and *Advances in Biological and Medical Physics*, an unprecedented diffusion of biological problems into the physical and mathematical journals and a great number of papers concerned with the utilization of physical techniques or modes of thought in physiological and clinical journals. We are familiar with this process as it occurred between the wars, when increasing numbers of chemists turned their attention to living processes and, in so doing, founded the independent discipline of biochemistry. What we are now observing represents the coming of age of biophysics.

The introduction of medical workers to biophysics is often *via* electronic instrumentation, a specialized branch of applied physics; this is a misfortune which often conceals from them the genuine assistance which may be obtained from the older science. They are presented with a collection of "black boxes" by poorly informed commercial agents who instruct them to connect the patient or the preparation to one end and a recorder to the other and to interpret the results in terms of the dial settings on the box. At worst this can lead to the confusion of a surgical team attempting multi-channel recording of every conceivable hæmodynamic variable in the operating theatre; at best it contributes little of itself to an understanding of the paraphysical living system to which the instruments have been connected. Since the last great war, electronics has produced dozens of new tools for the biologist and clinician, and for these we are most grateful; but tools remain tools, and are no substitute for thought.

The application of physical instruments to a biological problem is of little value, unless the physics of the system being studied is adequately understood and the characteristics of the instrument are precisely known and, above all, unless the hypothesis underlying the work is clearly stated and its consequences are logically deduced. Unless this is so, the application of a new instrument may degenerate into an aimless collection of data of questionable relevance and plentifully besprinkled with artefacts. The investigation, instead of being clarified by the judicious application of physical thought, is often confused and confounded by the indiscriminate importation of electronic engineering. Let us, therefore, set aside the art and science of instrumentation and consider the nature of physics proper. What is the central mode of thought in physics that has made it so successful in the study of inanimate matter? Is this mode of thought applicable to living material? If so, what branches of physics are of most value to us and in what areas of medical research may they be best applied? In attempting to answer these questions, we shall also be developing a definition of biophysics and forming views on its place in medical education and research.

PHYSICS AS AN AID TO THE STUDY OF INANIMATE MATTER

In the development of the natural sciences three stages may be discerned. The first stage consists of observing and describing phenomena, at first qualitatively, later with increasing quantitative precision. When a sufficient number of quantitative observations have been made, it is often possible to link them in a

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surprisingly simple relationship, which is then known as a natural or empirical law. Kepler, for instance, united Tycho Brahe's astronomical observations in three simple laws describing the motions of the planets about the sun. He made no assumptions about the underlying reasons for such motion.

The second step was taken when men first supplemented the information obtained by observation with that obtained through experiment. Passive observation of an occurrence is an inadequate and sporadic thing; much more information can be obtained if the occurrence is produced by the active intervention of the observer, at a time and under conditions convenient to him, while the accuracy of his observations will be much improved by the possibility of repetition. The body of information was greatly enlarged by the discovery of the experimental method; it could then, as before, be coordinated in natural laws. The bulk of empirical laws known to science have been produced to describe experimental results. Galileo's laws for falling bodies, Boyle's gas laws, Mendel's law of plant inheritance, all are laws derived from experimental results; it should be noted again that they suggest no underlying reasons for the phenomena observed—they merely describe them in a simple and generalized fashion.

The third and decisive step was taken by Newton, when he produced a mathematical theory of planetary motion. The gravitational law which he derived from this was of a new type. It was a theoretical and speculative statement about the nature and magnitudes of the forces acting between the sun and the planets, based on the assumption that the planets were subject to the same mechanical laws as applied to bodies on earth. The observations against which any such assumption might be tested were already unified in Kepler's natural laws. A new requirement had arisen, however—to deduce logically the consequences of his assumption in terms of the planetary motions to be expected, in order that these might be compared with the actual motions described by Kepler. It was in the development of mathematics as the logical bridge between assumption and consequence that the new step was taken. Speculation about the inner nature of things is as old as mankind. What was new was the framing of the speculation in a quantitative mathematical law, the deduction of the consequences by rigorous mathematical logic, and the comparison of these consequences with the natural laws describing the phenomena. This new type of law, the first law of mathematical physics, was able to link a much wider range of observations than was the experimental method alone. Such diverse phenomena as the fall of bodies to earth, the motion of the planets, the tides, the precession of the equinoxes, could now be explained in terms of one simple assumption.

We can now perceive the unique place of physics in the natural sciences; it is the only one in which the third stage, the mathematical development of theoretical laws, has been applied with outstanding success. In one field after another it has been possible to make speculative assumptions, to develop the mathematics necessary to draw logical deductions from these, and to test the resulting predictions by refined quantitative experimentation. Dirac defined the main object of physical science as the formulation of laws governing phenomena and the application of these laws to the discovery of new phenomena. It may be objected that this is equally the object of all sciences. If we understand "laws" to be empirical laws, the generalizations of observation, the objection can be sustained. But Dirac, as a theoretical physicist, is speaking of the speculative mathematical laws, which, with their related assumptions and deductions, form the body of his subject. It is still largely true that the successful formulation of these laws has been mainly carried out in physics. The new phenomena which may be discovered by the application of these laws need not, however, be purely physical phenomena. The subject of physical chemistry, for instance, largely consists of the application of theoretical physics to chemical problems and the resultant discovery of new chemical phenomena. We can similarly state the first essential in any definition of biophysics. It is that branch of science which attempts to apply the laws governing physical phenomena to the discovery of new phenomena in biology. Its main concern is the observation of physical principles at work in the living organism, and its basic assumption is that these principles are the same in animate as in inanimate matter.

Physics, then, would appear to have achieved its success by an ability to formulate general theoretical laws, to deduce the consequences of these rigorously, and to compare such consequences with a body of empirical knowledge built up by quantitative observation and experiment. The biophysicist will assume that a given physical law is equally binding in the living system which he is investigating. With suitable mathematical aids he will deduce the consequences of this assumption and compare them with experimental findings. If he is successful, he will have increased the range of phenomena explicable in terms of the basic

theoretical law and may also be able to predict the existence of new phenomena. In the history of physics, however, different types of physical theories have been developed, not all of which are of relevance to a given medical or biological problem. It will be helpful to distinguish some of these and to note the fields in which they have been of assistance to the living sciences.

PHYSICS IN THE STUDY OF LIVING MATERIAL

We first note that the early theories of physics were mechanical in nature; they depended on reference to such physical attributes as force, mass, momentum, stress and strain. This was a natural consequence of the early study of moving bodies, given impetus by the great mechanical achievements of the Industrial Revolution. In so far as animals may be regarded as lever-propelled metabolizing systems, studies of form and locomotion may be attempted by the methods of classical mechanics. This has already been done in the flight of insects, the locomotion of quadrupeds and the internal structure of animals, for instance. A systematic theory of propulsion has yet to appear, however, largely because the equations of motion of a series of linked levers present extraordinary mathematical difficulties. This is a common situation in the application of physics to biology.

Mathematical tools adequate to explore and develop the consequence of an assumption in the inanimate world often fail before the greater complexities of a living system. Further progress, then, awaits discovery of more powerful mathematical methods. Alternatively, the assumptions may be simplified and certain variables dismissed from the calculation, to bring it within the reach of mathematics already available. However, this approach must be made with great caution, lest we end up with an adequate theoretical description of a system too far removed from the living reality to be useful. The mechanistic theories, however, made their greatest impact in corpuscular form. Once it was assumed that the molecules of a gas were stable discrete entities interacting between themselves and the walls of a vessel according to the simple laws of mechanics, it was possible by straightforward mathematical deduction to link together the macroscopic properties of the gas in a unified explanation. This kinetic theory of gases was suggested by Bernoulli in the eighteenth century and worked out by Maxwell and Boltzmann in the nineteenth century. Amongst the properties deduced for a gas from this simple premise were some as yet unnoticed. In particular it was predicted that the viscosity of a gas would rise with temperature, a surprising result, soon verified experimentally. The theory was also applied, although with rather less success, to liquids and solutes. From the resulting body of experimental facts and theoretical construction we may draw heavily in studies of certain paraphysical subsystems of the human body. Gas flow in the airways of the lung, diffusion across the alveolar membrane and through the plasma, the fluid dynamics of the circulation, the diffusion of oxygen and metabolites into cells—these all require reference to kinetic theory for their adequate description. Before we leave the comfortably familiar images involved in the mechanistic stages of physical thought, it will be helpful to refer to concrete examples of their application to medical or biological research. The first is from a paper by D. L. Fry (1958), in which he utilizes physical knowledge about gas flow and about the mechanics of distensible tubes to explain certain properties of the human lung. He holds the degree of doctor of medicine, his approach is that of a physicist to a physiological problem, the result is biophysics. Dr. Fry considers a segment of bronchial tree between branchings. During expiration the pressure on the surface of the lung increases, and this increase is transmitted more or less uniformly throughout the parenchyma to the outside of the bronchial tree. However, inside any bronchial segment the pressure will be less than that outside, owing to the pressure drop associated with outward flow of the gas. There will thus be a net force acting perpendicularly across the bronchial wall and increasing in the direction of the flow. The diameter of a uniform elastic tube will, in these circumstances, decrease in the direction of flow. This causes a further pressure drop in the gas flowing in the tube and a further narrowing in the direction of flow. In such a system it is possible that an increased pressure drop along the tube may cause, not an increase, but a decrease in the rate of gas flow. This may be related to experimental curves in human subjects, which show that as intrapleural pressure rises, the volume flow rate rises, reaches a maximum and then declines. Were we to leave the matter at this level of qualitative supposition, it would be plausible but unproveable. Fry's contribution is to take from physics various experimental results, theoretical constructions and mathematical methods, and use them to deduce quantitative consequences from the original suggestion. He obtains support for this theoretical structure by experiments on a simple rubber model of the bronchial segment. He then applies it to the lung by

assuming that flow restriction takes place throughout the lung at the same level of bronchial branching, and shows that the curve of maximum flow rate versus volume should (*a*) be reproducible in any given subject, (*b*) be unaffected by variation in upper airway resistance, and (*c*) show a predicted change in shape with variation of the density or viscosity of the gas being breathed. The first two predictions are known to be correct, the third has yet to be tested. By fitting his equation to an experimental curve, it should be possible to determine the average compliance of the bronchial segments responsible for the flow limitation. As this compliance normally increases from carina to bronchiole, its value should indicate whether the flow-limiting segments are low or high in the tree. The values obtained for the other constants in his equation might be similarly instructive in tracing the progress of disease.

A paper such as this is a good example, then, of the application of physical laws to the discovery of new phenomena in medicine. It is not unduly concerned with instrumentation; none the less, the experimental work makes use of pressure and flow meters and requires a full understanding of their characteristics. It uses in its physics mechanical ideas or their development into the corpuscular form of gas kinetics, and utilizes mathematical techniques associated with the same period of development. So far as the medical research worker is concerned, this is the most useful field in physics from which to seek assistance. A knowledge of gas and liquid flow theory, of the physical laws of diffusion and of mechanical deformation, together with a minimum of the relevant mathematics, will open for him a wealth of new approaches to hæmodynamics, dye dilution methods, respiratory function, the quantization of clinical observation, surgical methods relating to the circulation, renal function and a host of others. The application of diffusion theory to the passage of gases through the alveolar membrane showed that no active process need be postulated; the same body of physical knowledge applied to the Malpighian tubule showed that selective active reabsorption had to be accepted if renal function was to be understood. However, in much of biology, physiology and biochemistry the greatest assistance has come from the use of concepts and methods drawn from later and more advanced levels of physical thought, in particular the concepts which were developed to describe adequately molecular and atomic events; before passing to them, we should briefly consider the development of field theories in physics.

Field theories differ from mechanistic theories in that they do not deal in mechanical categories. The difference is particularly marked in comparison with mechanistic theories of the corpuscular type; the field theory examines the continuous distribution of some physical condition throughout space rather than focusing attention on the discrete particles of the corpuscular theory. Two pre-conditions were required before field theories could become widely established in physics. The attention of physicists had to be drawn to the existence of non-mechanical physical categories, which was first done by Maxwell's discovery of the electromagnetic field, and the properties of partial differential equations, which are required for the description of continuous fields, had to be worked out. The methods of vector and tensor analysis which arose with this were not only essential to the further development of field theory, but could also be applied to the older mechanistic problems. We find, for instance, that although the basic assumptions of diffusion theory are justified by reference to kinetic theory, the mathematics by which many diffusion problems are tackled involve vector analysis and partial differential equations, techniques developed for use in field theory. We are, in fact, describing the diffusion of the solute or gas in terms of a continuous vector field of concentration gradients, and using field theory mathematics to investigate a basically corpuscular and mechanistic process.

This use of mathematics developed in one field of physics to tackle problems arising in another is common; it emphasizes the independent and versatile role of this symbolic logic. An example of medical relevance is furnished by M. G. Taylor's approach (1957) to the analysis of the arterial pulse wave. He pointed out that pulse waves recorded at different sites in the arterial tree differed considerably in shape, a phenomenon for which there is as yet no satisfying explanation. He proceeded, therefore, to a study of the oscillatory motion of a fluid in an elastic tube, as a precursor to a closer examination of the physiological system. To analyse this mechanical system he made use of equations developed to describe the passage of alternating current down an electric transmission line. With their help he was able to predict the phase velocity of pressure waves down the tube and the variation of this with the degree of occlusion at the end of the tube. He then set up a rubber tube and a pump to provide sinusoidal pressure pulsations and measured the velocity of the waves at different tube lengths and different degrees of occlusion. The results bore out the predictions

very closely, and showed that this borrowing of electrical mathematics was justified. They also made it clear that earlier attempts to explain the arterial pulse variations in terms of reflection and standing waves were inadequate. The way has thus been cleared for the application of this method of analysis to the greater complexities of the arterial tree. The methods and concepts of the mechanistic phase of physical thought are likely, then, to be of great value in investigating sub-systems of the human body, particularly if supplemented by the later mathematics developed in other branches of physics. But the most recent entry of physics into the sciences of life is occurring at a more fundamental level, that of atomic and molecular interactions. The physical and biological sciences have this level in common, the difference lying merely in the great molecular complexity characteristic of living material. Given the rapidly developing ability of the new quantum theories to investigate the more complex molecules, it is inevitable that physicists and physical chemists should seek to apply them in this most challenging field. Let us briefly consider the nature of these theories.

The necessity for the complex abstractions of quantum theory are best understood by harking back to the classical mechanistic view of the atom as a miniature solar system. This view was quickly found inadequate to furnish a precise explanation of the radiations emitted from an atom under the excitation of heat or electrical discharge. Bohr then suggested that certain limitations be placed upon the familiar mechanical laws in their application to atomic events; the electrons were not to be allowed the normal continuous range of energies, but to be confined to certain quantum states; the energy changes involved in abrupt transitions between such states were then related to the radiations emitted. This theory adequately explained the spectrum of radiations shown by such a simple system as the hydrogen atom, but became hopelessly complicated when the heavier atoms were considered, and, even with subsidiary quantum restrictions, failed to account with any precision for the spectra observed. Heisenberg then suggested a new approach, in which the positions, motions and orbits of the electrons were not mentioned, the aim being to connect only the observable magnitudes, the radiations, by appropriate mathematical methods. The development of the theory then proceeded to disclose by inference the mysterious mechanical laws of the atomic world. In so doing it led to the surprising conclusion that there are no exact microscopic mechanical laws, a conclusion which is most widely known in the form of Heisenberg's uncertainty principle. At the same time, de Broglie was developing a theory in which waves played a predominant part in guiding the atomic particles. De Broglie thought that the waves were physically real, but Schrodinger, in developing the implications of the theory, showed that they must in many cases be supposed to be propagated in multi-dimensional space, which precluded their physical reality. Finally, Schrodinger's wave theory was shown to be mathematically equivalent to Heisenberg's theory, and the combination came to be the basis upon which rests our present understanding of atomic and molecular events.

The outlook of quantum theory now pervades all of nuclear, atomic, molecular and solid state physics, and has in large part made possible the striking experimental and technological advances of recent decades. It has also taken away the simple and familiar model of the atom upon which a qualitative understanding of atomic events used to depend. A very wide range of observable phenomena are now linked together by an underlying network of intricate mathematical relationships, involving a degree of abstraction not previously used in scientific theories. But, as Dirac said, the task of physics is not the provision of pictures, but the formulation of laws governing phenomena, and in this the later and highly mathematical theories of physics have been outstandingly successful. To this success we owe the enormous contribution which is now being made on the molecular level to the living sciences. The configuration of proteins, the forces between macromolecules, the phenomena of energy and electron transfer in ordered biological structures, the production of radicals in photosynthesis, are all being actively investigated against a background of modern physical theory. It is unrealistic to expect the medical teacher or research worker to be informed in this field of biophysics. However, he should be aware that these fundamental studies are being pursued, he should have colleagues who can interpret the work to him, and he should be ready to take advantage in his own field of advances in knowledge made at the molecular level.

Some reference must now be made to the role of modern machines. The tasks performed by such machines, and the thinking which lies behind their design, are no longer purely mechanistic, in the sense of relying solely upon mechanical magnitudes. They are still, however, completely deterministic. If Event A is caused to occur, Event B inevitably follows, whether the two are linked by rods, electric

circuits or light beams. The mathematical aids to their design can therefore be drawn upon in the study of similarly deterministic systems in, for instance, the human nervous system. One of the most powerful of such aids is Boolean algebra, a system of symbolic logic particularly suited to the study of complicated assemblages of very simple units. It is much used in the design of digital computers, whose basic units are either " on " or " off ". It may equally be used in the examination of neural networks, when the individual neurone is either transmitting an action potential or is quiescent. Given a sufficiently complicated network, with the additional assistance of threshold effects and synaptic delay times, it is possible to simulate certain properties observed in the human nervous system. Indeed, there has been developed a formal method for constructing any complex net which has certain prescribed properties. It should be noted that this implies no assertion as to the anatomical existence of such a neural network. Its value lies in exploring the possible modes of action of nets known to exist, and in suggesting further experimental approaches. The same may be said of cybernetics, the science of self-correcting machines. In so far as it assists in the quantitative description of feedback mechanisms found to operate in the body, it can be most valuable; but in its attempts to understand the body as a mechanism, it must be disciplined by anatomical knowledge of what is there, and by physiological understanding of the lack of complete determinism in living processes.

To counterbalance the determinism of the machine designers, we have the recent development of statistical theories in the medical field. This is not to be confused with the widespread use of statistics in assessing experimental results. The latter is a technique for extracting the best conclusion from widely scattered results; the former is a method for predicting the behaviour of an assemblage of units, when the behaviour of each unit is in some respects random or indeterminate. This is a particularly difficult task when the behaviour of the unit is also heavily influenced by the state of those about it. A very pretty example is given by a recent theory of cortical organization.

It has been pointed out by Sholl (1956) that anatomical evidence makes it difficult to accept " switchboard " theories of organization in the cerebral cortex. Not only is the required specificity of connexion lacking, but the loss of function occasioned by the removal of portions of cortex is much less than such theories would seem to demand. Sholl's own description of cortical anatomy is a statistical one; aggregates of cells are described rather than specific neural networks, and the influences operating on a single cell are related to the number of other cell processes passing through its dentritic field. Cragg and Temperley (1954) have pointed out the analogy between this type of organization and the phenomena studied by statistical mechanics. In a substance where the interaction between neighbouring units is high, properties arise which are qualitatively different from those exhibited when interaction is low. Ferro-magnetism is exhibited in iron owing to the high degree of interaction between the constituent atoms. When the iron is heated sufficiently to destroy this interaction, the magnetic properties are lost. The principle may be demonstrated by setting a large number of small compass needles on a table. If these are sufficiently far apart, they will line up north and south. As they are more closely packed, their mutual influence overrides that of the earth's magnetic field, and groups of needles swing to a new orientation. A large assemblage of compasses will show many such domains, the total domain pattern being that which minimizes the free energy of the system. If any domain is disturbed by an external influence, its pattern will change and a wave of activity may be passed through the whole assemblage, thus changing the overall domain pattern. Cragg and Temperley develop the postulate that cooperative processes of this sort occur in some central nervous tissue, and proceed to make six deductions from it, each of which is in better agreement with observation than such deductions as can be made from rival hypotheses. Two of the deductions are of particular interest. The development of cooperative activity depends upon sufficient growth to bring about a certain critical density of neurones in the cortex; this may be related in the growing child with the abrupt onset of electroencephalographic patterns just before or after birth. The loss of function caused by destruction of cortical tissue would be expected to be roughly proportional to the amount of tissue destroyed, which is in accord with observation. The predictions must be quantitatively tested before the theory can be regarded as being established, and this requires the use of the physical theory to calculate the expected size and velocity of movement of the domains, and the measurement of these properties in a variety of cortical tissues. It is interesting to note that calculations of this type have been undertaken by R. L. Beurle and others at the Royal Radar Establishment of the British Ministry of Supply (1954).

We have given four examples of the influence of physical thought in the medical field: the application of physical laws and the accompanying theory, mainly of a mechanistic kind, to the discovery of new properties of certain systems in the body; the application of modern quantum theory to the molecules of living material; the study of the living body as a mechanism, by analogy with modern physical machines and using mathematics useful in their design; and the creation of a biological hypothesis by analogy with a physical hypothesis dealing with similarly organized material. These examples could be multiplied indefinitely, but they are sufficient to make our point.

The impact of physics in medicine is not confined to instrumentation. On the contrary, its greatest contribution is made when modes of physical thinking are applied to the biological problem. It is most successful when applying laws already formulated in physics to the discovery of new phenomena in biology; this is the true purpose of biophysics, often concealed from the medical worker by unfortunate experiences with "black boxes". Work of this kind is best done by pure physicists who have learnt their biology in post-graduate years. They must be disciplined to the vagaries and complexities of living material by working amongst biologists, but their highest skills must be physical.

This said, let us briefly discuss some of the problems of instrumentation. The traditional difficulty here is lack of understanding of the instrument by its user, and ignorance of the medical problem on the part of its designer. This gap may be bridged from either side. An electronics engineer or applied physicist may proceed to a training in physiology and the accumulation of experience in a medical school, or a medical graduate may spend some years learning physics and electronics. The former is usually the better, in that this primary training will produce a wider range of skills of instrumentation and will induce a constant urge to improve the admittedly poor precision of medical measurement. In clinical work, however, the physicist must be taught respect for the patient and the ethics of experimental interference. Neither of these people can be replaced by a technician, for this leads immediately to the familiar vicious circle. The doctor orders an instrument and a technician produces one to his specifications. When it is applied to the patient, the specification proves to have been inadequate; the recording is in dubious relationship with the quantity to be measured, while the latter has little relevance in terms of the physics of the system being investigated.

There is only one adequate way in which to develop an instrument for a medical investigation. The problem in its most general form must be taken by the medical worker to the physicist *cum* physiologist, who reads the literature and forms his own views on the value of the proposed research and the relevance of the suggested physical measurement. He will then discuss the matter with the medical people, inform them of the physics involved and, with the cynicism of his kind, demand to be convinced that theirs is the best mode of attack on the problem. When all the team are agreed on this and on the instruments required, he should design or purchase them, and then participate in the work of their application. While so doing, he should be taught more of the medical aspects of the work and should himself instruct his colleagues on the physical aspects. Given adequate facilities and technical assistance, such a person can both check the present tendency to over-instrumentation and ensure that genuinely necessary physical measurement is carried out competently and with an improved understanding of what is being measured.

If medical research in this country is to reap the benefit of both physical thought and physical instrumentation, ways must be found to attract pure and applied physicists into the hospitals and medical schools, and to encourage them, either before or after their appointment, to a study of physiology and other relevant medical sciences. The way forward, as in England and the U.S.A., is via the creation of small departments of biophysics or medical physics. The stimulus to this may be, for instance, a decision to make use of radioactive isotopes, or to purchase instruments for a cardio-vascular unit. It is false economy merely to buy a few instruments and employ an electronics technician. If the truly relevant measurements are to be made with accuracy, if money is not to be wasted on unsuitable instruments, if conclusions are to be accurately drawn, it is essential to engage a physicist with physiological experience and to give him adequate premises, equipment and technical assistance. As his medical interests develop he will wish to attempt some major advance in medical measurement or to undertake researches of his own in some paraphysical system of the body, and should be encouraged in this. He should be expected to read widely and to interpret to his colleagues biophysical advances of relevance to their work. He should be in close touch with the schools of physics and chemistry, and should be able to interest them in the biological and medical problems coming

to his notice. We cannot hope to produce overnight a flourishing school of mathematical biophysics, but we can help to foster the conditions for its emergence. And we can at least ensure that the exciting developments in biophysics do not pass us by for lack of people to interpret them.

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